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Review

Immunosuppressive and anti-inflammatory effects of cyclic AMP phosphodiesterase (PDE) type 4 inhibitors

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1. Introduction

It has been approximately a decade since early studies (Crummey et al., 1987; Torphy and Undem, 1991) first highlighted the anti-inflammatory and smooth muscle relaxant effects of cyclic AMP phosphodiesterase (PDE) 4 inhibitors that suggested their potential in the treatment of asthma. During the intervening period, many pharmaceutical companies have embarked on programs of work to identify their own PDE4 inhibitors, not only for the treatment of asthma, but also other chronic inflammatory diseases such as arthritis, chronic obstructive pulmonary disease (COPD), atopic dermatitis and multiple sclerosis (MS). At the time of writing (mid-1999), over 450 patents have been published and several compounds have been evaluated in the clinic, mostly for asthma. However, in general, the clinical results to date with PDE4 inhibitors in asthma have been disappointing and several development compounds have been discontinued. As well as this intensive activity on the pharmaceutical front, the last 10 years have witnessed great leaps forward in our understanding of the molecular biology and biochemistry of PDE4 as well as the pharmacology of PDE4 inhibitors, which may create opportunities for future therapeutic innovations.

The current article briefly reviews the molecular biology and biochemistry of PDE4 as well as the in vitro and in vivo data supporting the view that PDE4 inhibitors are potentially exciting novel drugs for the treatment of chronic inflammatory diseases. The reasons for the failure of PDE4 inhibitors in the clinic are addressed and the possibility that advances at the molecular level will translate into the identification of PDE4 inhibitors with greater therapeutic success is critically discussed.

2. Cyclic AMP cascade

Cyclic AMP is a ubiquitous second messenger that transduces intracellular signals initiated by many biologically active agents exerting their effects through activation of adenylyl cyclase. It plays an important role in the immune system, exerting generally suppressive effects on the functions of inflammatory and immunocompetent cells (Kammer, 1988). The only known way in which cyclic AMP exerts its biological actions is through binding to the regulatory subunit (R) of the heterodimeric cyclic AMP-dependent protein kinase (PKA) leading to dissociation of the catalytic subunit (C), which phosphorylates and changes the activities of target proteins, thereby altering cellular functions (Scott et al., 1991).

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Cyclic AMP levels are controlled by extrusion from the cell (Wiemer et al., 1982) or by cyclic AMP PDE, which is the only means of degrading the second messenger.

A complex picture of the cyclic AMP cascade has emerged over the past few years with the demonstration of multiple forms of adenylate cyclase (at least nine) (Sunahara et al., 1996), PKA (Faux and Scott, 1996) and cyclic AMP PDE (see below). Evidence for the segregation of G-coupled receptors to discrete regions of the plasma membrane of cells indicates that the pattern of cyclic AMP gradients generated within the cell in response to different extracellular stimuli may not be uniform (Houslay and Milligan, 1997). The intracellular cyclic AMP 'detecting' system is also spacially complex. Two regulatory subunits of PKA have been identified, one of which (RI) is exclusively cytosolic, whereas the other (RII) is targeted towards membranes in various intracellular locations through binding to a variety of A kinase anchoring proteins (AKAPs) (Faux and Scott, 1996). A picture thus emerges (Houslay and Milligan, 1997) of cyclic AMP synthesis and detection being a 'highly vectorial process' possessing distinctive regulatory features with PKA–RII serving to 'sample' cyclic AMP concentrations in distinct intracellular compartments. As discussed later on, there is now evidence that cyclic AMP PDE can also be targeted to different intracellular sites by anchoring proteins (APs). Thus, it is likely that different 'compartmentalized' cellular functions are regulated by spatially distinct adenylate cyclases, PKAs and cyclic AMP PDEs (Houslay and Milligan, 1997).

3. PDE isoenzyme family

Cyclic nucleotide PDEs (EC 3.1.4.17), discovered more than 30 years ago (Butcher and Sutherland, 1962), hydrolyse the phosphodiester bond of purine cyclic nucleotides (cyclic AMP, cyclic GMP) to their corresponding 5′-mononucleotides (5′-AMP, 5′-GMP), which do not activate cyclic nucleotide-dependent protein kinases. Multiple PDEs have been identified (Beavo et al., 1994). These isoenzymes differ in their substrate specificity, kinetic properties, responsiveness to endogenous regulators (Ca^{2+}/calmodulin, cyclic GMP), and susceptibility to inhibition by various compounds. Furthermore, it has been demonstrated that many cyclic nucleotide PDEs are separate gene products with multivariant regulatory (Ca^{2+}/calmodulin, cyclic GMP binding sites) and other ill-defined domains linked to highly conserved (> 60% amino acid identity) and homologous catalytic sequences (approximately 300 amino acids), which are located near the carboxyl terminus of the enzyme. Until recently, seven major families had been identified (Table 1). These families are designated by the Arabic numerals 1, 2, 3, 4, 5, 6 and 7 and correspond to Ca^{2+}/calmodulin-stimulated-, cyclic GMP-stimulated-, cyclic GMP-inhibited-, cyclic AMP-specific-, cyclic GMP-specific-, photoreceptor- and rolipram-insensitive (cyclic AMP-specific)-PDEs, respectively. Two further families, PDE8 and PDE9, have recently been identified from expressed sequence tags (ESTs) as part of the ongoing efforts to identify and sequence all known expressed genes in the human genome and through traditional cloning studies in murine tissues. PDE8 is a cyclic AMP-specific PDE which is resistant to inhibition by specific inhibitors of other isoenzymes as well as the non-selective inhibitor, 3-isobutyl-1-methylxanthine (IBMX) (Fischer et al., 1998a; Hayashi et al., 1998). PDE9 is highly specific for cyclic GMP but is only weakly inhibited by PDE5 inhibitors such as zaprinast and sildenafil (Fischer et al., 1998b). Murine homologues of PDE8 and PDE9 have also been cloned and characterized (Soderling et al., 1998a,b). The recently reported murine, dual-substrate PDE10 exhibits higher affinity for cyclic AMP (K_m: 0.05 μM) than cyclic GMP (K_m: 3.0 μM) (Soderling et al., 1999). In the future, it is likely that other families will be identified.

Members of one family share 20–25% sequence homology with members of another. Each family contains two or more related subfamilies (designated with a capital letter), which are derived from similar (70–90% homology) but distinct genes. Furthermore, several of the subfamilies have multiple members (designated with Arabic numerals) produced by alternative mRNA splicing or different start sites for translation of the protein. Thus, the accepted nomenclature for describing products of PDE4 genes is based on (1) the first two letters indicating the species (HS, *Homosapiens*; RN, *Rattus norvegicus*), followed by (2) PDE, for phosphodiesterase, (3) an

Table 1
Properties and selective inhibitors of cyclic nucleotide phosphodiesterase isoenzymes
Nomenclature based on Beavo et al. (1994).

PDE family	Isoenzyme	K_m (μM)		Subtypes
		Cyclic AMP	Cyclic GMP	
1	Ca^{2+}/CaM-stimulated	2–70	2–20	3
2	cGMP-stimulated	30–100	10–30	1
3	cGMP-inhibited	0.1–0.5	0.1–0.5	2
4	cAMP-specific	0.5–4.0	> 50	4
5	cAMP-specific	> 40	1.5	1
6	Photoreceptor	> 500	60	4
7	Rolipram-insensitive cAMP-specific	0.2	> 1000	1
8	IBMX-resistant, cAMP-specific PDE[a]	0.15	> 100	2?
9	High-affinity cGMP PDE[a]	230	0.07–017	?
10	Dual specificity PDE4[a]	0.05	3.0	?

[a] Recently identified, novel PDE isoenzymes for which no agreed family name has been designated.

Arabic numeral for the PDE family, (4) a single letter for the gene (A, B, C, D for each of the four PDE4 gene families), (5) an Arabic numeral for the splice variant and (6) a single letter for the report (Beavo et al., 1994).

4. Biochemistry and molecular biology of PDE4

4.1. Multiple PDE4 forms

Four human and rat PDE4 genes (PDE4A, B, C, D) have been identified and their locations on human and mouse chromosomes determined (Houslay et al., 1998) (Table 2, Fig. 1). Multiple splice variants exist for at least three of the four PDE4 genes (Houslay et al., 1998). The conservation of many individual mRNAs between human and rat demonstrates PDE4 to be a highly conserved multigene family. Sequence analysis has identified several highly conserved domains in PDE4 subtypes. The catalytic domain, which encompasses approximately 300 amino acids, is highly conserved in all PDE isoenzymes. There are two upstream conserved regions (UCRs 1 and 2) towards the N-terminal. PDEs that contain both UCRs are known as long forms, whereas those that lack the extreme N-terminal UCR-1 are known as short forms. UCR-1 (~ 55 residues) is separated from UCR-2

Table 2
Human PDE4 subtypes and splice variants identified to date

Subtype	Genebank name	Accession number	Chromosome locus	Amino acids
PDE4A	HSPDE4A1	U97584	19	611
	HSPDE4A4	L20965		886
PDE4B	HSPDE4B1	L20966	1p31	736
	HSPDE4B2	M97515/L12686/L20971		564
	HSPDE4B3	U85048		721
PDE4C	HSPDE4C1	Z46632[a]	19	712
	HSPDE4C4	U66346		791
PDE4D	HSPDE4D1	U50157/U79571	5q12	586
	HSPDE4D2	U50158/AF012074		508
	HSPDE4D3	L20970/U50159		673
	HSPDE4D4	L20969		810
	HSPDE4D5	AF012073		746

[a] Incorporates an artifact of cloning and has not been shown to encode an active enzyme. Four rat PDE4 genes have also been identified and many of the individual mRNAs are conserved between humans and rats. For a more exhaustive analysis of the PDE4 clones and isoforms, see Houslay et al. (1998).

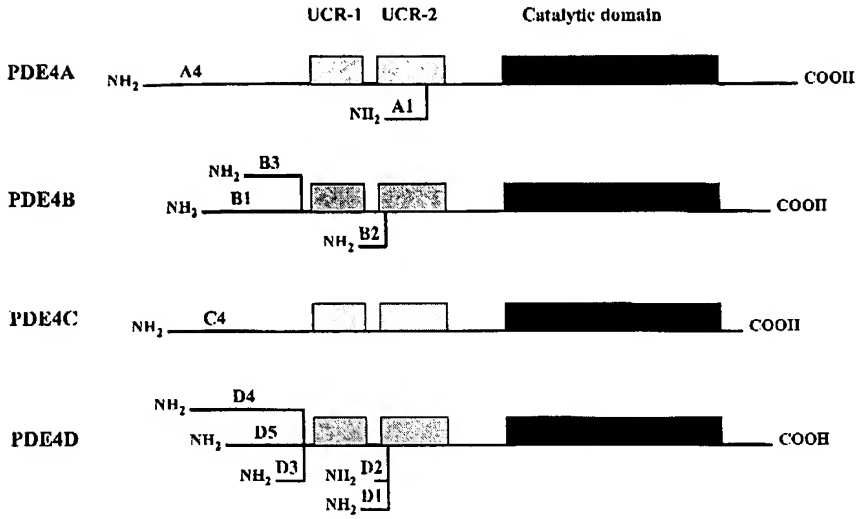

Fig. 1. PDE4 subtypes and splice variants. The positions of catalytic domain and upstream conserved regions (UCR-1 and UCR-2) are represented by the shaded boxes. Alternative mRNA splicing results in short forms in which UCR-1 is deleted and long forms with extended N-termini. For more exhaustive analysis of PDE4 clones, see Houslay et al. (1998).

(~ 74 residues) by a linker region (LR-1) of approximately 33 residues and UCR-2 is separated from the catalytic domain by LR-2, which is approximately 28 residues in length (Houslay et al., 1998) (see Fig. 1).

Partial genomic sequences have been published for short forms of rat PDE4B and PDE4D genes (Monaco et al., 1994). The human PDE4A gene structure, characterised with respect to both short- and long-form encoding cDNAs, is complex, spanning 49 kB with at least 17 exons (Houslay et al., 1998).

The cellular/tissue distribution of PDE4 subtypes A, B, C, D and their splice variants has been investigated at the mRNA level by reverse transcription polymerase chain reaction (RT-PCR) (using subtype and splice-variant-specific primers), Northern blotting and RNAse protection and at the protein level by immunoblotting (Houslay et al., 1998). There are several instances where mRNA levels do not correlate with protein expression patterns (Houslay et al., 1998). Each PDE4 gene has a distinct pattern of expression in tissues and cells (Engels et al., 1994; Houslay et al., 1998).

4.2. Properties of multiple PDE4 subtypes

PDE4 exhibits a high-affinity for cyclic AMP (K_m 0.5–4.0 μM) but has weak affinity for cyclic GMP ($K_m > 50$ μM) (Conti and Swinnen, 1990). Activity is dependent on critical histidine residues (Jin et al., 1992; Jacobitz et al., 1996), which may coordinate Zn^{2+} at the catalytic core as has been proposed for PDE5 (Francis et al., 1994). The catalytic activity of PDE4 is affected by events occurring at the N-terminal (e.g. post-translational modification, association with membranes or proteins — see below) (Houslay et al., 1998). How this occurs is uncertain, but interactions controlled by LR-1 between UCR-1 and UCR-2 via LR-1 have been proposed to induce conformational changes at the catalytic site via LR-2 (Houslay et al., 1998). Furthermore, PDE4 can associate with other proteins that can impact on the conformation of the catalytic site, possibly explaining why the same PDE4 construct in different expression systems exhibits different properties determined, perhaps, by the intracellular availability of protein 'partners' in which the recombinant PDE4 is being expressed (Houslay et al., 1998). UCR-1 and UCR-2 may be involved in homodimeric or heterodimeric interactions, which may also affect catalytic activity — evidence for PDE4 existing as either monomers or dimers has been reported (Houslay et al., 1998).

The subcellular localization of PDE4 subtypes is determined by amino acid sequences at the extreme

N-terminal of PDE4 subtypes. RNPDE4A1 is a short-form PDE4 splice variant that when expressed in COS-1 or COS-7 cells is found to be associated with membranes, particularly the Golgi fraction (Shakur et al., 1993). Membrane association is dependent on the N-terminal, tryptophan-rich 23 residues that contain two independent folding helical regions separated by a mobile region (Smith et al., 1996). It is uncertain how such a structure can insert into membranes (no lipid acylation sites are present), but APs may be involved (McPhee et al., 1995). Human and rat long form splice variants of the PDE4A gene, namely RNPDE4A5 and HSPDE4A4B, which contain proline-rich domains with PxxPxxR motifs in the N-terminal splice region (~256 amino acids), have been proposed to associate with membranes by binding to APs such as cortactin and fodrin which possess SH3 domains (O'Connell et al., 1996). Both PDE4 forms also interact with SH3 domains of the tyrosine kinases, src, fyn and lyn but not those of Grb2 or crk (O'Connell et al., 1996). Neither HSPDE4D4A (long form), nor the short form, RNPDE4A1, interacts with these SH3 motifs (O'Connell et al., 1996). The potential physiological role of such interactions is uncertain although their importance may be suggested by the fact that elevation of cyclic AMP opposes activation of inflammatory/immunocompetent cells in which src family kinases play important roles (Chan et al., 1994). PDE4B2, which possesses four potential N-terminal myristoylation sites, is found in association with the CD3ε chain of the T-cell receptor (TCR) of peripheral blood T-cells and becomes phosphorylated on tyrosine residues following CD3 ligation (Baroja et al., 1999). In contrast, PDE4B1, which is also found in T-cells but not in association with CD3ε, does not possess the N-terminal myristoylation sites and is not phosphorylated following CD3 ligation, although it, like PDE4B2, is tyrosine-phosphorylated when T-cells are exposed to pervanadate (Baroja et al., 1999). Short forms of PDE4D (HSPDE4D1 and HSPDE4D2) are essentially cytosolic, whereas long forms (HSPDE4D3, D4 and D5) are found distributed in the cytosolic and membrane fractions (Houslay et al., 1998). Different PDE4D variants are localized in discreet subcellular compartments of FRTL-5 thyroid cells and hormones cause the activation of these isoforms in a temporally and spatially dependent manner (Jin et al., 1998).

4.3. Regulation of PDE4

Agents that elevate cyclic AMP accumulation exert two types of control on cyclic AMP PDE — short- and long-term upregulation. Short-term activation of cyclic AMP PDE is one of several mechanisms to rapidly turn-off the cyclic AMP signal, whereas enzyme induction involving de novo protein synthesis, which has been observed in inflammatory cells (Torphy et al., 1992b, 1995; Verghese et al., 1995a; Manning et al., 1996), occurs as cells attempt to adapt to prolonged elevation of the second messenger.

The well-documented induction of cyclic AMP PDE occurs after prolonged (2 or more h) exposure of cells to cyclic AMP analogues or agents that elevate intracellular levels of the second messenger and is blocked by inhibitors of mRNA or protein synthesis (Torphy et al., 1992b). In monocytic cells, multiple subtypes are induced (Verghese et al., 1995a; Manning et al., 1996), leading to heterologous desensitization to prostaglandin (PG) E_2 (Torphy et al., 1995). Upon removal of the stimulus, cyclic AMP PDE activity decays slowly to basal levels over several hours (Torphy et al., 1992b).

In rat sertoli cells, increases in cyclic AMP upregulate 'short form' PDE4D1 and PDE4D2 but not PDE4D3 (Conti et al., 1995). Similar observations have been made in Jurkat T-cells (Erdogan and Houslay, 1997) and such data suggest that short — but not long — forms of PDE4D are under the control of a cyclic AMP responsive promoter (Vicini and Conti, 1997). A cyclic AMP-inducible intronic promoter controlling PDE4D1 and PDE4D2 transcription has been partially characterised (Vicini and Conti, 1997). Although these data point to a paradigm that short — but not long — PDE4 forms are upregulated by cyclic AMP, another study in peripheral blood T-cells (Seybold et al., 1998) refutes this since PDE4D3, as well as PDE4D1 and PDE4D2, mRNA transcripts are induced by exposure to 8-bromo cyclic AMP and stimuli of adenylate cyclase. In this study, transcripts of PDE4A4 and PDE3B forms were also upregulated in response to these stimuli in T-cells (Seybold et al., 1998). Although

elevated intracellular cyclic AMP in U937 cells increases expression of transcripts for PDE4A and PDE4B, expression of PDE4D3 appears to be decreased (Torphy et al., 1995).

Short-term activation of RNPDE4D3 occurs rapidly when cells are exposed to agents that stimulate adenylyl cyclase through PKA-dependent phosphorylation (Alvarez et al., 1995; Sette and Conti, 1996; Sette et al., 1994a,b). The short-term and long-term upregulation of PDE4 may have clinical implications, since it has been proposed that the increased cyclic AMP hydrolytic activity results in tolerance to β-adrenoceptor agonists after prolonged usage and may explain why these drugs are not anti-inflammatory (Giembycz, 1996). HSPDE4B2B is phosphorylated by mitogen-activated protein (MAP) kinase but without any apparent effect on activity (Lenhard et al., 1996) and potential phosphorylation sites for other protein kinases have also been identified on PDE4 subtypes (Houslay et al., 1998).

Activation of cyclic AMP PDE may be a common mechanism to facilitate the pro-inflammatory actions of cytokines and proliferative agents by lowering the levels of a second messenger known to antagonize their actions (e.g. see Koga et al., 1995). Increased cyclic AMP PDE activity is observed in mononuclear cells (monocytes and macrophages) in response to several pro-inflammatory stimuli, including histamine (Hanifin et al., 1985), lipopolysaccharide (LPS) (Okonogi et al., 1991; Ma et al., 1999) and cytokines such as interferon (IFN)-γ and interleukin (IL)-4 (Li et al., 1992, 1993). Exposure of human monocytes to LPS increases levels of PDE4B and anti-inflammatory cytokines, IL-4 and IL-10, reduce its expression, suggesting that this subtype plays an important role in regulating the activation state of this important inflammatory cell (Ma et al., 1999).

Activation of cyclic AMP PDE in peripheral blood lymphocytes and thymocytes has been observed in response to TCR ligation, PMA, concanavalin A (con A) and phytohemagglutinin (PHA) (Epstein and Hachisu, 1984; Valette et al., 1990; Michie et al., 1996, 1998). The increased PDE4 activity observed in thymocytes following TCR ligation with anti-CD3 antibody is blocked by staurosporine (non-selective kinase inhibitor), gentamycin (a non-selective tyrosine kinase inhibitor) and chelerythrin (a PKC inhibitor) implicating PKC and tyrosine kinases in the TCR-mediated induction of PDE4 (Michie et al., 1998). Induction of PDE4A, PDE4D as well as PDE1B1 in human peripheral blood lymphocytes occurs in response to the mitogenic stimulus, PHA, perhaps secondarily to elevation of cyclic AMP (Jiang et al., 1998). PDE4 and PDE3B activities are increased in human autoreactive $CD4^+$ T-lymphocyte clones specific for the immunodominant myelin basic protein (MBP) epitope following exposure to MBP (Ekholm et al., 1997).

PDE4 from monocytic cells and thymocytes is activated by cellular concentrations of phosphatidic acid (PA), which has been described as a T-cell mitogen whose levels are often elevated following activation of inflammatory cells (DiSanto and Heaslip, 1995; DiSanto et al., 1995; Savany et al., 1996). PA increases the V_{max} of the enzyme without affecting its K_m (DiSanto et al., 1995). Lysophosphatidic acids and phosphatidylserines also activate PDE4, whereas phosphatidylcholines, phosphatidylethanolamines and diacylglycerol do not (DiSanto et al., 1995). Concentrations of rolipram (10–100 nM) lower than are required to inhibit PDE4 can shift the PA concentration–response curve for PDE4 activation (DiSanto and Heaslip, 1995). PA activates 'long forms' of PDE4 (PDE4A5, PDE4B1, PDE4D3) but not 'short forms' (PDE4A1, PDE4B2, PDE4B2, PDE4D1, PDE4D2) (Elbawab et al., 1997; Nemoz et al., 1997).

4.4. High-affinity rolipram binding site

Until recently, rolipram was considered to be a relatively weak ($K_i \sim 0.5–1$ μM), competitive inhibitor of PDE4 (Conti and Swinnen, 1990); however, the existence of a stereoselective, high-affinity ($K_d \sim 1$ nM) binding site for rolipram has been appreciated since 1987 (Schneider et al., 1987). Its importance is indicated by the close correlation between the potency order of diverse cyclic AMP PDE inhibitors in displacing [^{3}H] rolipram from brain membranes and their central (Schmiechen et al., 1990) and peripheral (Harris et al., 1989; Souness and Scott, 1993; Barnette et al., 1995a,b, 1996a,b; Kelly et al., 1996) actions. Although this binding site is known to be associated with several PDE4 subtypes (Houslay et al., 1998), the rank potency order of compounds in inhibiting human recombinant

HSPDE4A catalytic activity and displacing [^{3}H] rolipram is markedly distinct (Torphy et al., 1992a). For example, the K_i of rolipram on catalysis is almost 100-fold greater than its $K_{i_{app}}$ in the binding assay (Torphy et al., 1992a). Furthermore, whereas the *R*-(−)-enantiomer of rolipram is only 3-fold more potent than the *S*-(+)-enantiomer in inhibiting cyclic AMP hydrolysis, 20-fold stereoselectivity is observed for binding (Torphy et al., 1992a). Although the nature of the high-affinity rolipram binding site has not been proven unambiguously, compelling evidence points to it as representing a distinct conformer of PDE4 with which rolipram interacts with high affinity (HA-PDE4) (Christensen et al., 1996; Jacobitz et al., 1996). Indeed, support for this comes from studies on HSPDE4B2, which binds rolipram at a single site with two different affinities dependent on the conformation of the enzyme (Rocque et al., 1997). There is evidence for a different cell/tissue distribution of HA-PDE4 and the form with which rolipram interacts with relatively low-affinity (> 100 nM; LA-PDE4) (Souness and Rao, 1997). High levels of rolipram binding have been measured in the brain (Schneider et al., 1987) and, although difficult to detect in peripheral tissues, its presence has been demonstrated in cell types (parietal cells, macrophages) in which functional responses to PDE inhibitors are better correlated with displacement of [^{3}H] rolipram from HARBS than inhibition of PDE4 catalytic activity (Barnette et al., 1995a,b; Kelly et al., 1996).

It has been suggested that the potency of rolipram against PDE4 is a sensitive monitor of the conformation of PDE4 (Houslay et al., 1998). Certainly, the IC_{50} value of rolipram against particular PDE4 species varies greatly depending on the cellular environment in which the enzyme is expressed and the intracellular compartment to which it is targeted (Houslay et al., 1998). Unlike rolipram, the potencies of other inhibitors, such as piclamilast (RP 73401), against PDE4 expressed in different expression systems are much more consistent (Jacobitz et al., 1996; Souness and Rao, 1997), suggesting that the two inhibitors interact differently with the enzyme. Rolipram exhibits simple competitive kinetics of inhibition of particular PDE4 species expressed in some expression systems whereas in others, complex, non-linear kinetics of inhibition is observed (Sullivan et al., 1994; Wilson et al., 1994). Expression of HSPDE4A4B in COS7 cells results in expression of cytosolic and particulate populations (Huston et al., 1997). Rolipram displays simple competitive inhibition against the cytosolic fraction but complex kinetics against the particulate fraction (Huston et al., 1997). These data strongly support the view that at least some PDE4 species can exist in different conformational states, which show different sensitivities to rolipram inhibition. The factors that determine the conformation of PDE4 are not fully understood, although it is known that post-translational modification (RNPDE4D3) and membrane association (HSPDE4A) (Alvarez et al., 1995; Sette et al., 1996; Huston et al., 1997) have marked pharmacological effects.

Phosphorylation of Ser^{54} in UCR-1 of RNPDE4D3 (long form) dramatically increases catalytic activity and the potency of rolipram (Alvarez et al., 1995; Sette et al., 1996). Whether PKA specifically activates the rolipram high-affinity component or switches a low-affinity form to an activated, high-affinity species is uncertain. It should be noted that Ser^{54} in the $RRES^{54}$ motif is a highly conserved residue in the UCR-1s of other long form PDE4 species, which have not been reported to be activated by PKA (Houslay et al., 1998). It is possible therefore that a priming event, as a result of protein–protein interaction or post-translational modification, which increases the potency of rolipram, is required for PKA to activate RNPDE4D3.

5. PDE4 inhibitor structural types

As shown in Fig. 2, over 450 PDE4 inhibitor patents have been published during the past 10 years, reflecting the huge interest in the therapeutic potential of this class of compounds. Most of the chemistry has been based on rolipram although xanthines (e.g. denbufylline, arofylline), nitroquazone and quinoline/napthaline (e.g. T-440) analogues have been widely evaluated in pre-clinical models (Palfreyman and Souness, 1996; Norman, 1998). Clinical data, mostly in asthma, have been reported on several of these compounds, namely, tibenalast

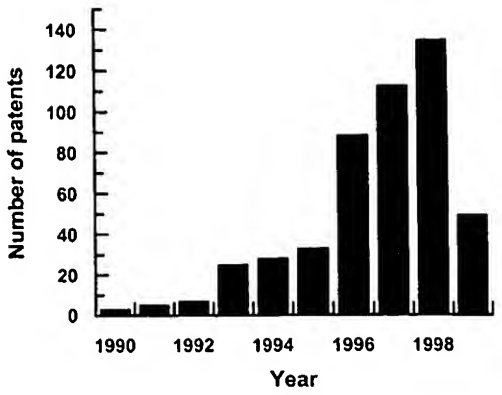

Fig. 2. PDE4 patents 1990–1999.

(LY 186655 —discontinued), piclamilast (RP 73401, Rhône-Poulenc Rorer — discontinued), CDP-840 (Celltech/Merck — discontinued), Ariflo (SB 207499-SKB phase III), arofylline (LAS-31025, Almirall phase III), atizoram (CP-80633, Pfizer phase II, atopic dermatitis) in inflammatory diseases (see below — for structures, see Fig. 3). Other compounds, such as D-4418 (Chiroscience/Schering-Plough — discontinued), D-22888 (Asta Medica — discontinued), filaminast (WAY PDA 641, American Home Products — discontinued), YM-58997 (Yamanouchi phase I?), V-11294 (Napp/Purdue phase I), CI-1018 (Jouveinal/Park-Davis phase I) and roflumilast (BY-217, Byk-Gulden phase III) are also reported to be (or have been) in the clinic (asthma), but no efficacy information has so far been divulged (for structures, see Fig. 4) (Norman, 1998; Montana et al., 1999; 'R&D Insight' Database, Adis International, UK; Pharmaprojects Database, PJP Publications, UK). CC-3025, (Celgene) a thalidomide analogue with PDE4 inhibitory activity, is reported to be in phase I clinical trials for rheumatoid arthritis (Norman, 1998). The poor clinical results with the majority of PDE4 inhibitors to date are possibly due to insufficient compound being administered as a consequence of dosing constraints imposed by side-effects (see below). To identify compounds with improved therapeutic ratios, a wide range of novel structures has been synthesised, including benzopyrazoles, benzimidazoles, benzofurans, diazepino-indoles, quinolines, quinolones and purines (Norman, 1998).

6. Anti-inflammatory effects of PDE4 inhibitors in vitro

PDE4 is widely distributed in cell types implicated in chronic inflammatory diseases such as asthma (Table 3). Its physiological importance is demonstrated by the wide-ranging suppression of many inflammatory/immunocompetent cell functional responses by PDE4 inhibitors (Table 4).

6.1. Granulocytes

Activation of mast cells and eosinophils is thought to play an important role in the pathogenesis of allergic disorders such as asthma. The release of

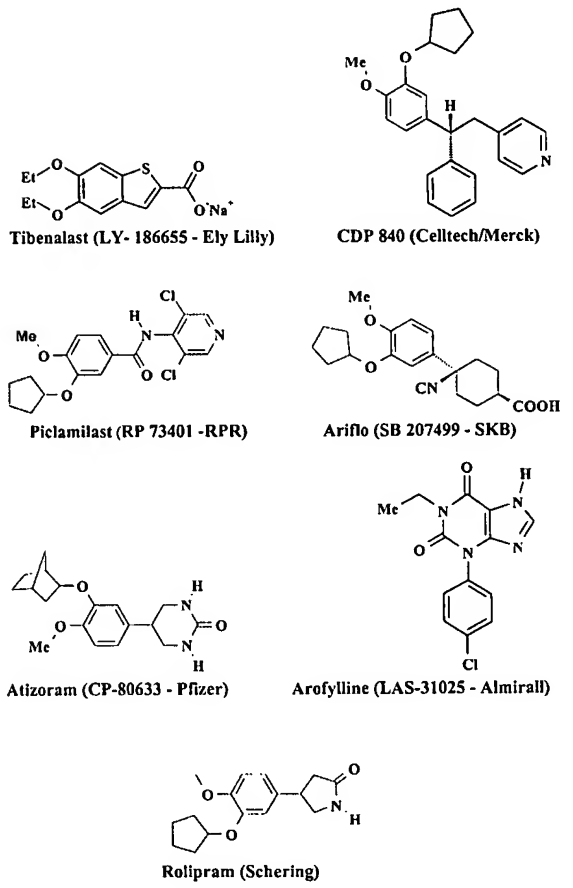

Fig. 3. Structures of PDE4-selective inhibitors for which clinical efficacy data in inflammatory diseases has been reported. The structure of rolipram is included for comparison.

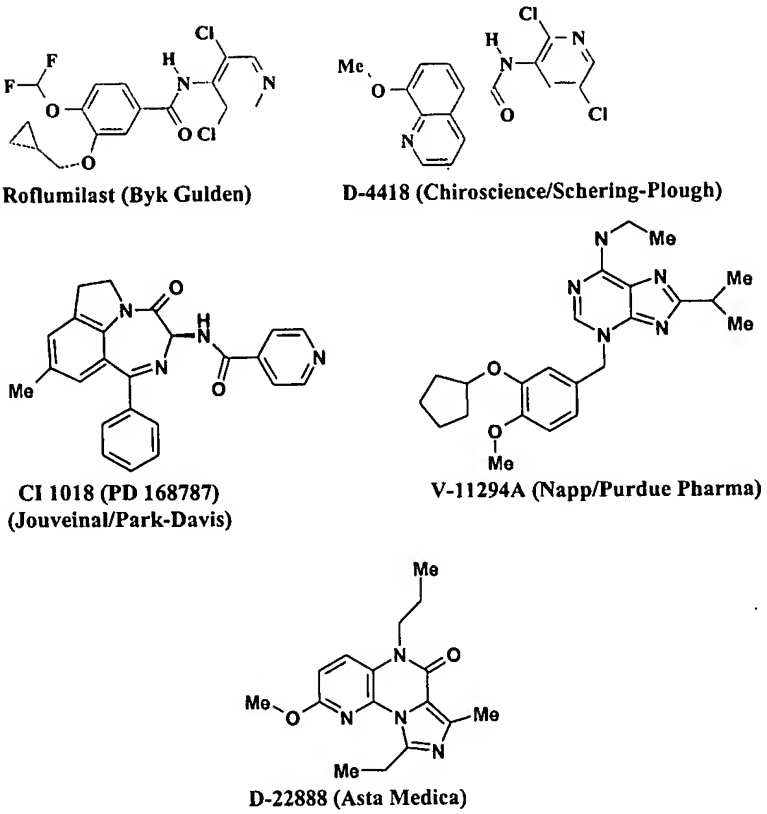

Fig. 4. Structures of recently divulged PDE4 inhibitors, which are reported to have entered pre-clinical or clinical development.

mediators such as histamine and leukotrienes (LTs) from sensitized mast cells is believed to be a primary cause of the acute bronchoconstriction in response to antigen exposure (Barnes et al., 1988) and release of cytokines such as IL-4 and tumour necrosis factor-alpha (TNF-α) may contribute to the inflammatory responses in asthmatic late-phase reactions (LAR) (Gordon et al., 1990; Schwart, 1994). The effects of

Table 3
Cyclic AMP PDE isoenzyme profiles in human inflammatory/immunocompetent cells

Cell type	PDE isoenzyme(s) present	Reference
Basophils	3, 4	Peachell et al., 1992
B-lymphocytes	3, 4, 7[a]	Gantner et al., 1998
Eosinophils	4	Dent et al., 1994
Epithelial cells	3, 4	Dent et al., 1998
Macrophage	3, 4	Tenor et al., 1995a,b
Mast cells	3, 4	Weston et al., 1997
Monocytes	4 (3?)	Torphy et al., 1992a,b; Souness et al., 1996
Neutrophils	4	Wright et al., 1990
T-lymphocytes	3, 4, 7	Robicsek et al., 1991; Tenor et al., 1995a,b; Giembycz et al., 1996

[a] PDE7-like activity reported.

Table 4
Effects of PDE4 inhibitors on human inflammatory/immunocompetent cells

Cell type	Suppressive effect	Reference
Basophils	Mediator release	Peachell et al., 1992
B-lymphocytes	IgE production	Cooper et al., 1985; Coqueret et al., 1997
Eosinophils	Superoxide generation	Giembycz et al., 1996
	Chemotaxis	Kaneko et al., 1995; Tenor et al., 1996
	Degranulation	Kita et al., 1991; Hatzelmann et al., 1995
	LTC_4 synthesis	Tenor et al., 1996
	CD11b expression	Berends et al., 1997
Macrophages	TNF-α release	Schudt et al., 1995
Monocytes	TNF-α release	Semmler et al., 1993; Souness et al., 1996
	LTB_4, cysteinyl LTs	Griswold et al., 1993
	IL-10 release (stimulation)	Suda et al., 1998
Neutrophils	Superoxide generation	Nielson et al., 1990
	Chemotaxis	Harvath et al., 1991
	Mediator release	Fonteh et al., 1993; Denis and Riendeau, 1999
	CD11b expression	Berends et al., 1997
	Degranulation	Derian et al., 1995; Barnette et al., 1996a,b, 1998
T-lymphocytes	Proliferation (H)	Essayan et al., 1994; Giembycz et al., 1996
	Th-1 cytokines (IL-2, IFNγ)	Essayan et al., 1995; Giembycz et al., 1996
	Th-2 cytokines (IL-4, IL-5, IL-13)	Essayan et al., 1997a,b
	GMCSF	Van Wauwe et al., 1995

PDE4 inhibitors on mast cells varies considerably depending on the tissue and species from which the cells are isolated. In murine bone-marrow-derived mast cells, rolipram inhibits antigen-induced LTC_4 release (Torphy and Undem, 1991); however, in functional studies on rat peritoneal mast cells, the PDE5 inhibitor, zaprinast, but not rolipram, suppresses mediator release (Frossard et al., 1981). PDE4 inhibitors have little effect on immunoglobulin (Ig) E-induced mediator release from human lung mast cells (Weston and Peachell, 1998; Weston et al., 1997). Similar lack of efficacy of PDE4 and PDE3 inhibitors on mediator release has been reported in cultured human cord blood-derived mast cells (Shichijo et al., 1997). However, in human peripheral blood basophils, rolipram inhibits antigen- and anti-IgE-induced mediator (histamine, LTC_4) release, an effect which is potentiated by SK&F 95654 and forskolin (Peachell et al., 1992).

Eosinophilia is a prominent pathological feature of allergic diseases and is thought to contribute to the airways damage observed in asthma (Kay, 1985; Barnes et al., 1988). The cytotoxic potential of the eosinophil derives from its ability to generate reactive oxygen species, peroxidase and cationic proteins (Kay, 1985; Barnes et al., 1988). Eosinophil products induce epithelial damage and increase airway reactivity in experimental animals in vivo (Barnes et al., 1988), fuelling speculation that these cells may contribute to the hyperresponsiveness observed in asthmatics. Several studies demonstrate that the cytotoxic potential of eosinophils is potently reduced by PDE4 inhibitors. They suppress the generation of reactive oxygen species in both human (Dent et al., 1991; Ezeamuzie and Alhage, 1998) and guinea-pig (Souness et al., 1991; Dent et al., 1991; Barnette et al., 1995a,b) eosinophils in response to particulate (serum-opsonized zymosan) and several soluble stimuli. In addition, LTB_4-induced release of thromboxane (Souness et al., 1994), major basic protein (MBP) and eosinophil cationic protein (ECP) (Kita et al., 1991; Souness et al., 1995) as well as complement factor — hrC5a — and platelet-activating factor (PAF)-stimulated release of LTC_4 and eosinophil-derived neurotoxin (EDN) (Hatzelmann et al., 1995; Tenor et al., 1996) are potently inhibited by PDE4 inhibitors. Flow cytometric analysis with monoclonal antibodies shows that rolipram suppresses PAF-induced CD11b (α_M subunit of Mac 1) expression on and L-selectin shedding from human

eosinophils and neutrophils (Berends et al., 1997). Rolipram also suppresses eotaxin — but not granulocyte macrophage-colony-stimulating factor (GM-CSF)-induced expression of CD11b on human eosinophils (Santamaria et al., 1997; Momose et al., 1998), lending support to the contention that PDE4 inhibitors can suppress extravasation of eosinophils through an effect on rolling adhesion. Further evidence for actions on adhesion molecules is provided by the demonstration that rolipram synergizes with PGE_2 in suppressing the CD18 (β_2-subunit of Mac-1)-dependent aggregation of guinea-pig eosinophils in response to PAF and C5a (Teixeira et al., 1996), and data from an eosinophilic cell line (EoL-1) demonstrate that cyclic AMP downregulates expression of very late antigen $(VLA)_4$ (Jung et al., 1994). The eosinophil chemotactic responses to formyl-methionyl-leucyl-phenylalanine (fMLP), LTD_4, PAF, eotaxin and other soluble stimuli are potently inhibited by PDE4 inhibitors (Cohan et al., 1992; Kaneko et al., 1995; Alves et al., 1996, 1997; Tenor et al., 1996; Santamaria et al., 1997). Finally, several studies suggest that PDE4 inhibitors inhibit IL-5-induced survival of human eosinophils (Kubota, 1996; Ohta et al., 1996; Momose et al., 1998).

Neutrophils are important in host defense, accumulating rapidly at sites of injury or infection. The aberrant release of proteases and reactive oxygen species can lead to extensive tissue damage (Badwey and Karnovsky, 1980). Neutrophils play important roles in acute and chronic inflammatory diseases, although their functions in allergic conditions are unclear. PDE4 inhibitors suppress several neutrophil responses, including degranulation (Derian et al., 1995; Barnette et al., 1996a,b, 1997), superoxide anion generation (Nielson et al., 1990; Barnette et al., 1998), release of IL-8 (Au et al., 1998), phagocytosis (Bessler et al., 1986; Au et al., 1998), adhesion (Derian et al., 1995), adhesion molecule (CD11b/CD18/L-selectin) expression (Derian et al., 1995; Berends et al., 1997), chemotaxis (Harvath et al., 1991), survival (Aoshiba et al., 1995) and mediator production (Fonteh et al., 1993; Denis and Riendeau, 1999). Rolipram also synergizes with adenosine in blocking fMLP-induced oxidative activity in TNF-α-primed neutrophils and inhibits the adherence to a fibrinogen-coated surface and the oxidative burst elicited by TNF-α (Sullivan et al., 1995). Suppression of neutrophil function appears to be linked to inhibition of phospholipases A and D (Nakashima et al., 1995) as well as enhanced resequestration of cytosolic Ca^{2+} into intracellular stores (Anderson et al., 1998).

6.2. Mononuclear phagocytes

Tissue macrophages (several types) and their circulating precursors, blood monocytes, play three distinct but interrelated functions (Adams and Hamilton, 1984). They recognize and remove inflammatory stimuli, act as antigen-presenting cells, and release several pro-inflammatory factors, includinghydrolytic enzymes, growth factors, lipid mediators, reactive oxygen species and cytokines. The capacity of mononuclear phagocytes to remove inflammatory stimuli determines whether an inflammatory response progresses to overt expression of disease (Adams and Hamilton, 1984). Inappropriate activation of mononuclear phagocytes is thought to play a role in several chronic inflammatory diseases, notably rheumatoid arthritis (RA), where joint-invading monocytes release pro-inflammatory, tissue-destructive cytokines, and COPD, where macrophage-derived elastolytic enzymes contribute to destruction of the lung parenchyma (see below). Suppression by PDE4 inhibitors of arachidonic acid breakdown (Godfrey et al., 1987; Schad and Schudt, 1993), phagocytosis (Bessler et al., 1986) and production of reactive oxygen species (Lim et al., 1983; Bessler et al., 1986; Turner et al., 1993; Takei et al., 1998) has been reported. In contrast to the relatively weak inhibition of these functions, the generation of TNF-α by both monocytes (Molnar-Kimber et al., 1993; Semmler et al., 1993; Probhakar et al., 1994; Seldon et al., 1995; Verghese et al., 1995b; Greten et al., 1996; Souness et al., 1996; Barnette et al., 1998; Kleinman et al., 1998; Suda et al., 1998) and macrophages (Schad and Schudt, 1993; Schudt et al., 1995) is exquisitely sensitive to the inhibitory effects of PDE4 inhibitors. PDE3 inhibitors exert little effect on LPS-induced TNF-α generation in monocytes in which PDE4 is the predominant cyclic AMP hydrolyzing isoenzyme (Molnar-Kimber et al., 1993). However, differentiation of human monocytes to macrophages alters the PDE profile with an augmentation of PDE3 (and PDE1) activities and a decline

in PDE4 (Gantner et al., 1997c). Furthermore, in macrophages derived from the peripheral blood monocytes, a combination of both PDE3 and PDE4 inhibitors is required to maximally (40–50%) suppress the TNF-α response (Gantner et al., 1997a).

The mechanism by which PDE4 inhibitors suppress TNF-α release is uncertain although an effect on mRNA expression occurs (Probhakar et al., 1994; Souness et al., 1996). Inhibitory effects of PDE4 inhibitors on TNF-α production at the transcriptional and post-transcriptional levels have been suggested (Probhakar et al., 1994). The anti-inflammatory cytokine, IL-10, whose release from monocytic phagocytes is stimulated by PDE4 inhibitors, has been causally linked to the suppression of TNF-α generation, possibly through inhibition of NFκB transcriptional activation (Wang et al., 1995; Ollivier et al., 1996; Suda et al., 1998) although this has been called into question (Seldon et al., 1998). In murine peritoneal macrophages, the inhibition of LPS-induced IL-6 release by rolipram and Ro 20–1724 is completely abrogated by an anti-IL-10 neutralizing antibody (Kambayashi et al., 1995). In comparison with their effects on TNF-α, PDE4 inhibitors exert little or no effect on the release of Il-1β and some reports indicate little effect on IL-6 (Molnar-Kimber et al., 1993; Probhakar et al., 1994). However, studies in murine macrophages show that rolipram potently inhibits LPS-induced release of IL-12, a cytokine which switches T-cells towards a T-helper (Th)-1 developmental pathway and suppresses Th-2 responses (Liang et al., 1998).

6.3. T-lymphocytes and B-lymphocytes

T-cells release several cytokines which are, at least in part, responsible for coordinating the cellular responses in inflammatory disorders such as asthma and arthritis (Bochner et al., 1994). Studies in mice (Mosmann et al., 1986) and humans (Romagnani, 1991) have suggested that CD-4 positive Th lymphocytes can be functionally categorized into two groups based on the cytokines they elaborate: the Th-1 subtype is defined by its restricted cytokine production of IL-2, IFN-γ and TNF-β. These cells mediate delayed-type hypersensitivity reactions, are the major isolates from non-atopic donors, and are implicated in autoimmune diseases (Cher and Mosmann, 1987). The Th-2 subtype releases IL-4, IL-5, and IL-10 and constitutes the major isolates from atopic donors. In cognate and non-cognate interactions with B-lymphocytes, Th-2 cells support IgE synthesis (Del Prete et al., 1988; Parronchi et al., 1990).

PDE4 inhibitors inhibit anti-CD3 plus anti-CD28- and *Staphylococcal* enterotoxin A-stimulated proliferation of T-cells from Balb/c mouse spleens (Wancio et al., 1995; Souness et al., 1997) and also suppress PHA- and anti-CD3 stimulated proliferation of human peripheral blood T-lymphocytes (Robicsek et al., 1991; Giembycz et al., 1996). In purified human $CD4^+/CD8^+$ T-cells, incomplete inhibition of proliferation is observed with rolipram alone; however, a much greater suppression of [^{3}H] thymidine incorporation occurs in combination with the PDE3 inhibitor, CI-930 (Robicsek et al., 1991). Rolipram and cilostamide (PDE3 inhibitor) suppress the proliferative responses and cytokine release in MBP- or other myelin protein-autoreactive $CD4^+$ T-cells (Sommer et al., 1995; Ekholm et al., 1997). Since it is believed that many autoimmune diseases are, at least partially, mediated by autoreactive $CD4^+$ T-cells, these observations may have important implications, not only for the treatment of MS but also for other autoimmune disorders. Although combinations of PDE4 and PDE3 inhibitors appear to be sufficient to totally suppress anti-CD3 plus anti-CD28-induced proliferation and cytokine release, a recent study employing anti-sense oligonucleotides implicates PDE7 in T-cell activation induced by the same stimuli (Li et al., 1999).

A number of studies have demonstrated that cyclic AMP PDE inhibitors suppress the release from T-cells of IL-2, a major T-cell mitogen and activator of the immune system (Averill et al., 1988; Lewis et al., 1993; Giembycz et al., 1996; Souness et al., 1997). It is unlikely that this is the predominant mechanism by which PDE4 inhibitors exert their effects on [^{3}H] thymidine incorporation into T-lymphocytes since potency differences between PDE4 suppression of IL-2 and proliferation have been documented (Lewis et al., 1993; Giembycz et al., 1996). Furthermore, when a combination of phorbol myristate acetate (PMA) and ionomycin is used as comitogens, rolipram inhibits IL-2 generation but not proliferation (Giembycz et al., 1996). Elevation of cyclic AMP appears to have multiple inhibitory effects on

T-cell proliferation by blocking phospholipase Cγ1-induced phosphatidyl inositol (4,5) bisphosphate hydrolysis and inositol phosphate accumulation (Park et al., 1992; Tamir and Isakov, 1994), Ca^{2+} mobilization (Van Tits et al., 1991), phosphorylation of the TCR (Patel et al., 1987), IL-2 receptor expression (Krause and Deutsch, 1991) and IL-2 transcription possibly through effects on transcription factors such as NFAT, NFκB and AP-1 (Chen and Rothenberg, 1994; Tamir and Isakov, 1994; Hsueh and Lai, 1995; Tsurata et al., 1995).

PDE4 inhibitors suppress both Th-1 and Th-2 responses. The majority of early studies suggested that agents that elevate cyclic AMP, including PDE inhibitors, are more effective suppressors of Th-1 cytokines (IL-2, IFN-γ) than Th-2 elaborated cytokines (IL-4, IL-5) (Munoz et al., 1990; Novak and Rothenberg, 1990; Betz and Fox, 1991; Van der Poow-Kraan et al., 1992; Lee et al., 1993). Indeed, rolipram synergizes with the PDE3 inhibitor motapizone to increase IL-5 production from anti-CD3-treated D10.G4.1 (D10) Th-2 cells while the release of IL-4 is minimally affected (Schmidt et al., 1995). However, rolipram is more effective in blocking the proliferation of peripheral blood mononuclear cells (PBMCs) elicited by ragweed (Th-2) antigen than tetanus toxoid (Th-1) antigen (Essayan et al., 1994, 1995) and RT-PCR shows attenuation of IL-5 and interferon-γ (IFN-γ), but not IL-4, gene expression following allergen provocation (Essayan et al., 1995). Proliferative responses in human Th-2 antigen-specific T-cell clones are also more sensitive to the inhibitory effects of rolipram than Th-1 antigen-specific clones and this is mirrored in the suppression of expression of IL-4, IL-5 and IFN-γ (mRNA and protein) in the respective clones (Essayan et al., 1997a). In peripheral blood lymphocytes stimulated with PHA, rolipram and nitraquazone are 3–6-fold more potent in reducing production of IL-5 than IL-2, GM-CSF and IFN-γ (Van Wauwe et al., 1995). Filaminast suppresses the generation of IL-4 induced by anti-CD3 from a T-cell line derived from atopic donors (Crocker et al., 1994) and T-440 inhibits the release of IL-5, IL-4 and IL-2 from PBMCs (Kaminuma et al., 1996b,c). Expression and production of IL-13 in a T-cell clone from a ragweed (Th-2 antigen) allergic, asthmatic patient are also inhibited by rolipram with effects being demonstrated at the mRNA and protein levels (Essayan et al., 1997b). Suppression of IL-4 release with PDE4 inhibitors has also been observed in vivo (Griswold et al., 1998).

Less is known about the effects of PDE4 inhibitors on the B-cell responses. Early studies (Cooper et al., 1985) demonstrated that Ro 20–1724 inhibits the spontaneous IgE generation by PBMCs from individuals with atopic dermatitis; however, this effect is probably indirect via monocytes (Cooper et al., 1985). A more recent publication (Coqueret et al., 1997) supported this conclusion in demonstrating that the PDE4 inhibitors, rolipram and Ro 20–1724, inhibit IL-4-induced IgE production by PBMCs but not by purified lymphocytes. Interestingly, studies on $CD19^+$ B-lymphocytes purified from human peripheral blood demonstrate that LPS plus IL-4 induced proliferation is enhanced by rolipram and analogues of cyclic AMP (Gantner et al., 1998).

6.4. Endothelial and epithelial cells

Endothelial cells act as a tight barrier to prevent inflammatory cell and plasma protein infiltration from the microvasculature into tissues; however, inflammatory stimuli increase permeability and adhesivity of endothelial cells, allowing inflammatory/immunocompetent cells and plasma proteins to penetrate into sites of inflammation. Rolipram reduces hyperpermeability of endothelial monolayers (Suttorp et al., 1993, 1996a,b,c), an effect that may explain the inhibition by PDE4 inhibitors of pulmonary capillary ischemia–reperfusion edema (Ramard et al., 1994), PAF-induced microvascular permeability in an isolated rat lung model (Noel et al., 1995), and of microvascular leakage in guinea-pig airways (Ortiz et al., 1992; Raeburn and Karlsson, 1993). PDE2 and PDE3, as well as PDE4, may play a role in regulating endothelial permeability (Suttorp et al., 1993, 1996a,b,c). Studies in individual, intact capillaries from post-capillary venules in the mesentery of pithed frogs suggest that PDE4 inhibitors affect microvascular permeability by regulating the number of tight junction strands between endothelial cells (Adamson et al., 1998).

PDE4 inhibitors reduce the expression of adhesion molecules on the surface of endothelial cells, possibly by inhibiting NFκB-mediated transcription

(Ollivier et al., 1996). For example, rolipram, in combination with forskolin or cholera toxin, inhibits TNF-α-, PMA- and LPS-induced expression of E-selectin (Pober et al., 1993; Morandini et al., 1996). Intercellular adhesion molecule (ICAM)-1 expression does not appear to be affected by cyclic AMP and effects on vascular cell adhesion molecule (VCAM)-1 expression are variable (Pober et al., 1993; Morandini et al., 1996; Ollivier et al., 1996). In human lung microvascular endothelial cells, rolipram alone exerts little effect on TNF-α-induced adhesion molecule expression but, in combination with ORG 9935 (PDE3 inhibitor), suppresses expression of E-selectin and VCAM-1 but not ICAM-1 (Blease et al., 1998). One possible consequence of the downregulation of adhesion molecules by PDE4 inhibitors is the inhibition of transendothelial migration of lymphocytes, but not monocytes (Lidington et al., 1996).

As well as acting to protect tissues from the external environment and transporting electrolytes, epithelial cells act as a source of pro-inflammatory proteins, particularly in the airways. In addition to releasing mediators and chemotactic chemokines such as IL-8, epithelial cells appear to be capable of elaborating GM-CSF and IL-5, which promote the production, survival and activation of eosinophils. Studies performed to date show unimpressive effects of PDE4 inhibitors on epithelial cytokine release. In A549 and in primary human epithelial cells, rolipram at high concentrations (50 μM) only partially suppresses the IL-1β-induced release of GM-CSF, whereas the PDE3-selective inhibitor, ORG 9935, and the non-selective IBMX almost completely ablate the response (Wright et al., 1998). In other studies with selective PDE4 inhibitors, increased or decreased secretory responses have been observed (Dent et al., 1998; Fuhrmann et al., 1999). Interestingly, there is evidence that agents elevating cyclic AMP, including PDE4 inhibitors, have cytoprotective effects in epithelial cells (Ozawa et al., 1995; Dowling et al., 1997). For example, rolipram protects human nasal epithelial cells infected with *Pseudomonas aeruginosa* (Dowling et al., 1997). It is noteworthy that in guinea-pigs, rolipram, T-440 and theophylline have been reported to protect against the epithelial damage caused by ozone exposure (Matsubara et al., 1997).

6.5. Inhibition of excitatory non-cholinergic neurotransmission

Stimulation of bronchial C-fibres induces bronchoconstriction and inflammation, by means of a central reflex pathway and local release of the sensory neuropeptides, including substance P and neurokinin A (Qian et al., 1994). Studies on responses in guinea-pig bronchi to electrical field stimulation in vitro demonstrate that rolipram and Ro 20–1724, but not siguazodan, dramatically inhibit non-cholinergic contractions, suggesting a reduction in the release of pro-inflammatory peptides from C-fibre endings (Qian et al., 1994; Undem et al., 1994). Since these neuropeptides increase microvascular permeability, mediator release, inflammatory cell recruitment and mucus secretion as well as contracting bronchial smooth muscle, this property of PDE4 inhibitors may have important implications for their in vivo activities. Indeed, evidence for this is provided by the inhibition of vagally mediated non-cholinergic bronchoconstriction in guinea-pigs by CDP-840, a selective PDE4 inhibitor which is a poor bronchodilator in vivo (Holbrook et al., 1996).

6.6. Other cell types

PDE4 inhibitors inhibit the release of pro-inflammatory cytokines from cells implicated in several CNS inflammatory pathologies. For example, in LPS-stimulated murine microglia, PDE4 inhibitors suppress the release of TNF-α, IL-1β and IL-6 but increase production of the anti-inflammatory IL-10 (Yoshikawa et al., 1999). Rolipram potentiates the suppressive effect of forskolin on IL-1β- and TNF-α-induced ICAM-1 and VCAM-1 expression in astrocytes (Ballestas and Benveniste, 1997). The effect of rolipram on TNF-α-induced VCAM-1 expression is, at least in part, due to enhanced degradation of mRNA although this does not appear to be the mechanism by which elevated cyclic AMP downregulates TNF-α-induced ICAM-1 or IL-1β-induced VCAM-1 or ICAM-1 expression (Ballestas and Benveniste, 1997). It is noteworthy that IFN-γ and LPS reduce cyclic AMP levels in microglial cells and IFN-γ (but not LPS) suppresses the second messenger in astrocytes, suggesting that this may be one mechanism involved in the pro-inflammatory effects

of these agents (Patrizio et al., 1995). Rolipram reverses these effects of LPS and IFN-γ, indicating that activation of PDE4 is responsible for the decrease in cyclic AMP (Patrizio et al., 1995). Inhibition of superoxide generation and proliferation of mesangial cells by PDE4 and PDE3 inhibitors have led to the suggestion that they may be of value in treating glomerulonephritis (Chini et al., 1997).

7. Potential of PDE4 inhibitors in chronic obstructive pulmonary disease (COPD)

While most pharmaceutical companies in the PDE4 field have targeted asthma, recent clinical results with the SmithKline-Beecham compound, Ariflo, suggest considerable potential for PDE4 inhibitors in the treatment of COPD. This section presents the preclinical data suggesting potential of PDE4 inhibitors in airways inflammatory diseases and clinical data in asthmatic and COPD patients. Although the potential utility of PDE4 inhibitors in treating these airway disorders is due to their direct relaxant actions on airway smooth muscle as well as anti-inflammatory effects (Souness and Giembycz, 1994), this topic is beyond the scope of the current review. Bronchoconstriction will only be mentioned in the context of antigen responses whose suppression by PDE4 inhibitors is likely to be due to inhibition of mediator release.

The asthmatic airway is characterized by a mucosal inflammation, which appears to correlate with the severity of the disease and has been causally linked to the major symptoms of variable airflow obstruction and airway hyperresponsiveness (Barnes et al., 1988). Increased numbers of mast cells, eosinophils and lymphocytes are a major pathological feature of asthma (Barnes et al., 1988). The generation of mediators and cytokines from these cells initiates and fuels the airway inflammation, while the release of cytotoxic proteins, particularly from eosinophils, induces epithelial damage and may contribute to pulmonary hyperreactivity (Barnes et al., 1988). In allergic asthma, these processes are driven by immunoglubulin (Ig) E following pulmonary exposure to one or more allergens (Barnes et al., 1988). Exudation of plasma (containing mediators, chemoattractant agents and pro-inflammatory proteins) into the airway lumen also occurs through a process known as microvascular leakage (Persson, 1988). This is thought to enhance airway inflammation and bronchial constriction, leading to sloughing of the epithelium, impairment of mucociliary transport, narrowing of small airways, and mucus plug formation (Persson, 1988).

COPD is a common, although poorly understood, disorder encompassing bronchitis, emphysema and chronic severe asthma. It is characterized by irreversible airflow obstruction, increases in airway inflammatory cell numbers (predominantly alveolar macrophages and neutrophils), and elastolytic destruction of the lung parenchyma leading to loss of elasticity and excess mucous cell hyperplasia (Barnes, 1998). It has recently become a major focus for drug discovery within the pharmaceutical industry. The disease is predominantly, although not exclusively, linked to cigarette smoking (Barnes, 1998).

PDE4 inhibitors show efficacy in several preclinical models of airway inflammation, which suggest that they have potential in the treatment not only of asthma but also COPD (Table 5).

7.1. Inflammatory cell accumulation

Antigen exposure of sensitized animals leads to the accumulation of eosinophils and, in some cases, neutrophils in the lungs (Sanjar et al., 1990; Underwood et al., 1993; Raeburn et al., 1994; Turner et al., 1994; Kaminuma et al., 1997a,b). Selective PDE4 inhibitors or mixed PDE3/4 inhibitors administered by a variety of routes (per orem (p.o.), intraperitoneal (i.p.), subcutaneous (s.c.)) reduce antigen-induced eosinophilia in the BAL fluid and lung tissue of guinea-pigs, Brown Norway rats and monkeys (Turner et al., 1994, 1996; Howell et al., 1995; Gozzard et al., 1996; Hughes et al., 1996; Ortiz et al., 1996; Kaminuma et al., 1997a; Underwood et al., 1998). This is also seen following administration of micronized, dry powder formulations of rolipram or piclamilast directly into the airways of guinea-pigs (Raeburn et al., 1994). A variety of mediators, cytokines and chemokines, and chemoattractants for eosinophils are produced (Bochner et al., 1994), and several studies demonstrate that PDE4 and PDE3/4 inhibitors attenuate the eosinophilia induced in guinea-pig lungs by PAF, IL-5, IL-3, GM-CSF, LTD_4

Table 5
Actions of PDE4 inhibitors in pre-clinical inflammation models
MVL — microvascular leakage.

Disease	Model	Species	Reference
Airways inflammation	Antigen-induced eosinophilia	Guinea-pig	Underwood et al., 1993; Raeburn et al., 1994
		Monkey	Turner et al., 1994
		Rabbit	Gozzard et al., 1996
		Rat	Howell et al., 1995
	Mediator-induced eosinophilia	Guinea-pig	Lagente et al., 1994
	Cytokine-induced eosinophilia	Guinea-pig	Lagente et al., 1995
	Antigen-induced neutrophilia	Mouse	Kaminuma et al., 1997b
	LPS-induced neutrophilia	Mouse	De Moraes et al., 1998
		Rat	Escofier et al., 1999
	Antigen-induced hyperreactivity	Guinea-pig	Danahay and Broadley, 1997, 1998
		Monkey	Turner et al., 1994
	Ozone-induced hyperreactivity	Guinea-pig	Matsubara et al., 1997
	PAF-induced hyperreactivity	Guinea-pig	Raeburn et al., 1994
	LPS-induced hyperreactivity	Guinea-pig	Uno et al., 1998
	Mediator-induced MVL	Guinea-pig	Ortiz et al., 1993; Raeburn et al., 1994
	LPS-induced MVL	Guinea-pig	Howell and Jenkins, 1994
		Mouse	Miotla et al., 1998
	IL-2-induced MVL	Rat	Rabinovici et al., 1996
	Antigen-induced bronchospasm	Guinea-pig	Underwood et al., 1993; Raeburn et al., 1994; Danahay and Broadley, 1998
		Monkey	Turner et al., 1994
		Rabbit	Gozzard et al., 1996
		Rat	Raeburn et al., 1994
Arthritis	Adjuvant-induced arthritis	Rat	Sekut et al., 1995
	Type II collagen-induced arthritis	Mouse	Nyman et al., 1997; Ross et al., 1997
	Streptococcal cell wall-induced arthritis	Rat	Souness and Foster, 1998
Multiple sclerosis	EAE	Monkey	Genain et al., 1995
		Mouse	Sommer et al., 1997
		Rat	Sommer et al., 1995
Skin inflammation	Antigen-induced skin eosinophilia	Guinea-pig	Cooper et al., 1999
	Antigen-induced edema	Guinea-pig	Newbold et al., 1999
	Passive cutaneous anaphylaxis	Guinea-pig	Davies and Evans, 1973
Others	T-cell mitogen-induced liver failure	Mouse	Gantner et al., 1997a,b,c
	LPS-induced endotoxic shock	Mouse	Sekut et al., 1995
	Autoimmune insulitis and diabetes	NOD mouse	Liang et al., 1998

and TNF-α (Sanjar et al., 1990; Kips et al., 1993a; Lagente et al., 1994, 1995; Hughes et al., 1996). Furthermore, PDE4 inhibitors suppress endotoxin-induced inflammatory cell accumulation and edema in the lungs of guinea-pigs, an effect which may be mediated by suppression of TNF-α release into the BAL fluid (Kips et al., 1993a,b; De Moraes et al., 1998; Escofier et al., 1999). Inflammatory cell accumulation and acute lung injury (assessed by extravascular albumin accumulation) in mice exposed to a combination of LPS and zymosan are also reduced by rolipram although the effect appears to be independent of TNF-α (Miotla et al., 1998; Underwood et al., 1998; Gordon et al., 1999).

7.2. Bronchial hyperreactivity (BHR)

Selective PDE4 inhibitors as well as mixed PDE3/4 inhibitors suppress BHR to bronchoconstrictor agents induced by antigen and other stimuli in animal models (Santing et al., 1994; Danahay and Broadley, 1997). In conscious, unrestrained guinea pigs, i.p. administration of rolipram, or of ORG 20241 (PDE3/4 inhibitor) and theophylline, attenu-

ates hyperreactivity after both the early and late reactions (Santing et al., 1994); however, in another study (Sanjar et al., 1990), the mixed PDE3/4 inhibitor, benafentrine, and theophylline were ineffective in reducing PAF-induced BHR, although eosinophilia was substantially attenuated. Rolipram, administered by s.c. injection, although ineffective in blocking antigen-induced bronchospasm, significantly reduces BHR and eosinophilia in cynomolgus monkeys following repeat antigen challenge (Turner et al., 1994). Piclamilast, administered via the i.v. route to anaesthetized guinea-pigs, inhibits PAF-induced BHR to bombesin (Raeburn et al., 1994), and CDP-840 (i.p.) is as effective as budesonide in abolishing the antigen-induced increase in responsiveness to inhaled histamine in neonatally immunised rabbits (Hughes et al., 1996). CDP-840 and rolipram also inhibit ozone-elicited airway hyperresponsiveness to spasmogens in guinea-pigs (Holbrook et al., 1996; Matsubara et al., 1997). LPS-induced BHR in rats, in which TNF-α has been causally implicated, is inhibited by zardaverine, a mixed PDE3/4 inhibitor (Pauwels et al., 1990; Kips et al., 1992, 1993a,b). PDE4 inhibitors also suppress LPS-induced hyperreactivity in guinea-pigs (Uno et al., 1998).

7.3. Inhibition of antigen-induced bronchoconstriction

Suppression of antigen-induced bronchospasm by PDE4 inhibitors has been demonstrated in a variety of species (Underwood et al., 1993, 1997; Howell et al., 1993, 1995; Gozzard et al., 1996; Holbrook et al., 1996; Hughes et al., 1996; Kaminuma et al., 1996a; Jones et al., 1998; Montana et al., 1999). This effect of PDE4 inhibitors appears to be predominantly through an action on mediator release. For example, rolipram, administered i.v. to sensitized guinea-pigs, inhibits antigen — but not LTD_4 — or histamine-induced bronchoconstiction, suggesting that it exerts its inhibitory influence at the level of mast cells and, perhaps, basophils rather than by relaxing bronchial smooth muscle (Underwood et al., 1993). Ariflo is also more effective in suppressing bronchospasm induced by antigen than that elicited by mediators, again suggesting that it exerts its effect predominantly via mast cells (Underwood et al., 1998). Similarly, suppression of antigen-induced bronchospasm in squirrel monkey by CDP-840 also appears to be through an action on mediator release (Jones et al., 1998). Although anti-inflammatory activity and suppression of antigen-evoked responses are routinely observed with rolipram administered via the oral route, suppression of the bronchoconstriction elicited by several contractile agonists in guinea-pigs is not always observed (Underwood et al., 1993; Turner et al., 1994; Holbrook et al., 1996). Indeed, in cynomolgus monkeys, even antigen-induced bronchoconstriction is not inhibited by s.c. delivery of rolipram, although hyperreactivity and eosinophilia are suppressed (Turner et al., 1994). It should be noted that, in contrast to the variable effects observed when administered orally, rolipram and other PDE4 inhibitors suppress the bronchospasm evoked by a number of contractile agonists when administered to guinea-pigs as aerosols or dry powders directly into the airways (Raeburn et al., 1994). Interestingly, PDE4 inhibitors are particularly effective in blocking bronchospasm induced by LTD_4 (Raeburn et al., 1994; Underwood et al., 1998).

7.4. Microvascular leakage

The endothelium of post-capillary venules normally constitutes a tight barrier to blood cells and plasma proteins; however, during inflammatory episodes, endothelial cells contract in response to the release of mediators such as tachykinins, PAF and histamine following antigen challenge (Persson, 1988), leaving gaps through which cells and high molecular weight molecules can escape into the surrounding tissues. The increased microvascular permeability allows plasma proteins, including complement and blood clotting factors with pro-inflammatory potential, to escape and the accompanying fluid exudation causes edema (Persson, 1988).

Rolipram inhibits allergen-induced microvascular leakage into the lungs of sensitized guinea-pigs (Raeburn et al., 1991). Furthermore, rolipram administered via the oral, intratracheal or intravenous routes, markedly attenuates microvascular leakage into the small and large airways tissue as well as into the BAL fluid (Ortiz et al., 1992, 1996; Raeburn and Karlsson, 1993). In addition to the antigen-induced response, PDE4 inhibitors suppress leakage induced by several other stimuli, including histamine (Ortiz

et al., 1993; Raeburn et al., 1994), PAF (Ortiz et al., 1993; Raeburn and Karlsson, 1993), LPS (Howell et al., 1995) and LPS plus zymosan (Miotla et al., 1998). Rolipram, when administered i.p., inhibits IL-2-induced lung injury (assessed by protein accumulation in BAL fluid) in rats, an effect that appears to be mediated through inhibition of locally generated TNF-α and the subsequent reduction in the expression of TNF-α-stimulated cytokines (IL-1β, IL-6, cytokine-induced neutrophil chemoattractant) and adhesion molecules (E-selectin) (Rabinovici et al., 1996). Since IL-2- and LPS-induced microvascular lung injury are used as an experimental model for adult respiratory distress syndrome (ARDS), PDE4 inhibitors might be useful in the treatment of this pulmonary disease (Feuerstein et al., 1995).

7.5. Clinical results in asthma patients

Clinical efficacy of selective PDE4 inhibitors in asthma generally has been disappointing (see Table 6). An early single dose study with tibenalast (LY 186655, 150 mg, p.o.), a weak inhibitor, demonstrated a slight improvement in FEV_1 (Israel et al., 1988) and a study in asthmatic subjects with ibudilast, a compound which exhibits moderate PDE4 inhibitory activity (Souness et al., 1994), demonstrates suppression of BHR (Kawasaki et al., 1992). In allergen-challenged asthmatics, orally administered CDP-840 (15 mg b.i.d. for 10 days) significantly inhibited the late asthmatic response (Harbinson et al., 1997), but in other studies (histamine hyperreactivity and single dose exposure to asthmatic patients), was inactive (Harbinson et al., 1997). The inference drawn from these studies was that CDP-840 provided no immediate relief of symptoms and the compound was discontinued. Disappointing results have also been observed in a recent clinical study with inhaled piclamilast, which failed to modify FEV_1 following single dose administration (200 or 800 μg — RPR data on file) to moderate asth-

Table 6
Reported clinical experience with PDE4-selective and dual PDE3/4 inhibitors in inflammatory diseases
Compounds administered orally except where indicated. FEV_1 — forced expiratory volume in 1 min; CRP — C-reactive protein; LAR — late asthmatic response to antigen. Structures of PDE4 inhibitors are shown in Fig. 3 and dual PDE3/4 inhibitors in Fig. 5.

Compound	Disease	Dose	Effect observed	Reference
Tibenalast (LY 186655)	Asthma	150 mg	Slight improvement in FEV_1	Israel et al., 1988
Piclamilast (RP 73401)	Asthma	200–800 μg[a]	No effect	DeBrito et al., 1997
	Arthritis	400 μg, t.i.d.	Slight suppression of CRP and IL-6; improvement in symptoms	Chikanza et al., 1996
Arofylline (LAS-31025)	Asthma	20 mg	Improved FEV_1	Norman, 1999
CDP-840	Asthma	15 mg, b.i.d.	Suppression of LAR	Harbinson et al., 1997
Ariflo (SB 207499)	Asthma	10–15 mg, b.i.d.	Improved FEV_1; protection in exercise-induced asthma	Brown, 1999
	COPD	10–15 mg, b.i.d	Improved FEV_1	Brown, 1999
Atizoram (CP-80633)	Asthma	?	No published results	
	Atopic dermatitis	0.5% ointment[b]	Suppression of inflammatory parameters	Hanifin et al., 1996
Ibudilast[c]	Asthma	20 mg, b.i.d.	Suppression of hypersensitivity to histamine	Kawasaki et al., 1992
Zardaverine[c]	Asthma	6 mg[a]	Modest bronchodilation	Brunee et al., 1992
	COPD	1.5–6 mg[a]	No effect	Ukena et al., 1995
Benafentrine[c] (Ah 21–132)	Asthma	2–24 mg[a]	Slight bronchodilation in normal volunteers by inhalation; no effect when administered i.v. or p.o.	Foster et al., 1992

[a] Compounds administered by inhalation.
[b] Compounds administered topically to the skin.
[c] Dual PDE3/4 inhibitors.

matics (DeBrito et al., 1997). In phase II trials, Ariflo (10 mg, b.i.d.) showed efficacy in exercise-induced asthma (Brown, 1999) and a dose of 15 mg has been reported to improve respiratory function in patients with asthma who are not adequately controlled by inhaled corticosteroids (Compton et al., 1999a). Treatment with Ariflo for 6 weeks resulted in a 160-ml improvement in trough FEV_1 from placebo. Preliminary (6-week) data from a phase III trial involving over 300 patients with asthma showed that Ariflo (15 mg b.i.d.) markedly improved FEV_1 compared to placebo (Compton et al., 1999a).

Of the other rolipram-like structures, atizoram and filaminast have been withdrawn from development for asthma probably because of side-effects in the clinic (Norman, 1998). The most interesting of the remainder is the xanthine analogue, arofylline, which is now in phase III clinical trials (Norman, 1999). The only clinical data reported are from a small phase II study (16 patients with mild asthma) in which a significant increase in FEV_1 was observed with arofylline (20 mg) 1 h after administration (Norman, 1999). Little or no information is available on the development of other PDE4 structural classes, although it is known that clinical studies with several compounds are ongoing.

Debate still centres around the issue of whether selective PDE4 inhibitors or dual PDE3/4 inhibitors will be more efficacious in the treatment of asthma. That dual PDE3/4 inhibitors are more effective bronchorelaxants than selective PDE4 inhibitors is generally acknowledged (Souness and Giembycz, 1994; Underwood et al., 1994). There is little or no in vivo data from animal models of inflammation, however, to support the view that PDE3/4 inhibitors are more powerful anti-inflammatory agents than selective PDE4 inhibitors (Underwood et al., 1994). The bronchodilatory activity of two dual PDE3/4 inhibitors, benafentrine (AH 21–132) and zardaverine (for structures, see Fig. 5), has been evaluated in the clinic. When administered to normal volunteers by inhalation, benafentrine caused bronchodilation but was inactive when administered orally or intravenously (Foster et al., 1992). Zardaverine produced slight bronchodilation when administered by inhalation to asthmatics (Brunnee et al., 1992), but was inactive in patients with COPD (Ukena et al., 1995). Although the major concern of such mixed inhibitors

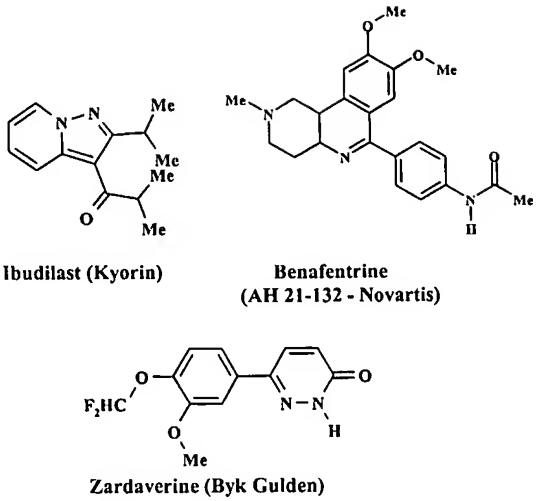

Fig. 5. Structures of dual PDE3/4 inhibitors for which clinical efficacy data in inflammatory diseases have been reported.

is their potential for causing cardiovascular side-effects (hypotension, increased cardiac contractility), the major problems observed in the clinic with zardaverine are those associated with inhibition of PDE4, namely nausea and emesis (Brunnee et al., 1992) (see below).

7.6. Clinical results in COPD patients

Recent results from a phase II trial in patients with COPD have demonstrated that treatment with Ariflo (15mg b.i.d. for 6 weeks) increases FEV_1 and forced vital capacity (FVC) by 11% and 7% over baseline, respectively. Ariflo treatment also improves peak expiratory flow rate (PEFR) by 25.3 l/min, exertional dyspnea, rescue bronchodilator use, as well as resting and post-exercise SaO_2 (Compton et al., 1999b). In the same clinical trial, Ariflo (10 and 15 mg) improved quality of life in COPD patients (Compton et al., 1999c). Adverse effects (e.g. headache) in these studies were reported as being mild to moderate in intensity and resolved by the end of the study (Brown, 1999). This suggests that Ariflo may be better tolerated in COPD patients than in asthma patients in whom nausea and vomiting is reported to occur when administered in 15 or 20 mg doses (Brown, 1999). Phase III clinical trials in COPD patients are ongoing and the results are awaited with interest.

8. Potential of PDE4 inhibitors in arthritis

RA is a painful, crippling systemic autoimmune disease of uncertain cause characterized by inflammation and progressive destruction of synovial joints (Pincus and Callahan, 1993). T-cells and monocytes, which are abundant in the RA synovium, play an important role in the disease. IL-1β and TNF-α are believed to play pivotal roles in causing the tissue damage in the RA joint and, in experimental models, administration of TNF-α directly into the rheumatoid joint initiates synovitis directly (Henderson and Pettifer, 1989). Current research is firmly focussed upon slowing (or halting) disease progression rather than modifying the accompanying symptoms and there is considerable interest in the treatment of RA through suppressing pro-inflammatory cytokines, particularly TNF-α (Badger and Lee, 1997). Thus, agents that elevate cyclic AMP, including PDE4 inhibitors that can suppress TNF-α release, may have potential in RA (Badger and Lee, 1997).

8.1. Preclinical models

Early rat adjuvant-induced arthritis studies (Bonta et al., 1978) with a combination of PGE_1 and theophylline were the first to indicate that cyclic AMP PDE inhibitors have anti-arthritis actions. Information in a Kyorin patent indicates that ibudilast reduces acute and chronic edema in adjuvant-induced arthritis (Irikura et al., 1987). More recently, rolipram has been shown to ameliorate adjuvant arthritis in rats (Sekut et al., 1995) and collagen II-induced arthritis (CIA) in rats and mice (Nyman et al., 1997; Ross et al., 1997). Rhône-Poulenc Rorer compounds, piclamilast and RPR 109026, cause suppression of symptoms in the CIA model in DBA/1Lac J ($H2^q$) mice equivalent to the suppression observed with an anti-TNF-α antibody (TN3-19.12, Genzyme) (Souness and Foster, 1998). Morphometric analysis of joints reveals that histological parameters (joint destruction, synovitis, erosion and fibrosis) are significantly improved by piclamilast and RPR 109026 (Souness and Foster, 1998). Similar suppression of disease symptoms by piclamilast is observed in the *Streptococcal* cell wall (SCW)-induced arthritis model in Lewis rats (Souness and Foster, 1998). Once again, joint swelling is suppressed and histomorphometric analysis reveals decreased areas of pannus and synovitis as well as a reduced absorption index (Souness and Foster, 1998). Several potential mechanisms may underpin the anti-arthritic actions of PDE4 inhibitors. These include inhibiting TNF-α release, increasing IL-10 release or suppressing functions of T-lymphocytes as well as direct, protective effects on cartilage and bone (Souness and Foster, 1998). Although stimulation of the hypothalamic–pituitary–adrenal axis (HPA) in rodents has previously been suggested as a possible mechanism by which PDE4 inhibitors exert their anti-inflammatory effects (Kumari et al., 1997), this is unlikely to account for the effects observed in SCW arthritis. For example, piclamilast ameliorates disease severity in SCW-induced arthritis in Lewis rats, a rat strain whose susceptibility to this disease has been attributed to a defective HPA response, without affecting either ACTH or corticosterone levels (Souness and Foster, 1998).

8.2. Clinical results in RA patients

RA patients treated with piclamilast (400 μg t.i.d., 4 weeks) in a small clinical study showed a positive trend in respect of serum concentrations of IL-6 and CRP although the corresponding levels of TNF-α and IL-1 were unaffected (Chikanza et al., 1996). Patients reported some improvement in symptoms. The administration of higher doses of piclamilast was prohibited by side-effects (Souness and Foster, 1998). A member of a series of structural analogues of thalidomide, which possess PDE4 inhibitory activity known as SelCIDs (selective cytokine inhibitory drugs, Celgene; CC-3052), is reported to be in development for RA (Norman, 1998; Souness and Foster, 1998).

9. Potential of PDE4 inhibitors in MS

Because of the numerous reports indicating that PDE4 participates in the biochemical processes controlling the behaviour of mammals and lower organisms, the potential of PDE4 inhibitors in treating various psychotic disorders was identified and early

compounds such as rolipram and denbufylline were developed for diseases such as depression and dementia (Palfreyman and Souness, 1996). Indeed, rolipram is still being developed by Meiji Seika in Japan for post-shock depression (Norman, 1998). More recently, interest has switched towards CNS indications where inflammation is thought to contribute to the pathology of the disease. As well as suppressing cytokine production and adhesion molecule expression in inflammatory cells in brain (Ballestas and Benveniste, 1997; Buttini et al., 1997; Yoshikawa et al., 1999), PDE4 inhibitors protect against blood–brain barrier disruption and are neuroprotective (Hulley et al., 1995; Kato et al., 1995; Uchiyama-Tsuyuki et al., 1996; Block et al., 1997; Yamashita et al., 1997; Belayev et al., 1998). Thus, patients with CNS diseases in which neuronal damage occurs as a consequence of an underlying inflammation, such as Alzheimer's disease, Parkinson's disease and stroke, may be responsive to PDE4 inhibitor therapy. Particular attention has focused on the development of PDE4 inhibitors for MS for which impressive efficacy in animal models has been reported (Dinter et al., 1997).

MS is an autoimmune disease characterized histopathologically by multiple foci of inflammation and demyelination disseminated throughout the brain, spinal cord and optic nerves (Hoban, 1998). The disease is highly variable between and within individuals over time and can adopt a progressive relapsing–remitting time course (Hoban, 1998). Early in the course of MS, inflammation (including immune cell infiltrates) and edema may occur, whereas in later stages of the disease, chronic demyelination, oligodendrocyte loss, astrogliosis and axonal damage are prominent (Hoban, 1998).

9.1. Preclinical models (experimental allergic encephalomyelitis, EAE)

In EAE in Lewis rats and marmosets, a model of MS in which TNF-α and MBP autoreactive T-cells have been implicated, PDE4 inhibitors, such as rolipram and ibudilast, are effective in reducing symptoms (Genain et al., 1995; Sommer et al., 1995, 1997; Fujimoto et al., 1999). In the relapsing–remitting mouse EAE model, administration of rolipram reduces clinical signs of EAE during the initial episode of the disease and in subsequent relapses (Sommer et al., 1997). This is accompanied by a marked reduction of demyelination and inflammation throughout the CNS and suppressed gene expression of pro-inflammatory cytokines in brain (Sommer et al., 1997). A principal mechanisms by which rolipram exerts these effects appears to be through suppression of Th-1 cytokine release, although the encephalitogenic potential of MBP-specific T-cells is not impaired (Sommer et al., 1997).

At the time of writing, there is no indication that a PDE4-selective inhibitor has been tested in MS patients.

10. Potential of PDE4 inhibitors in dermatological disorders

Although the level of interest in PDE4 inhibitors as potential treatments for dermatological disorders has fallen a long way short of that directed at respiratory disorders, compounds have been evaluated with some success in patients with dermatological complaints such as atopic dermatitis (AD) and psoriasis (Sawiski et al., 1979; Hanifin, 1991; Hanifin et al., 1996). AD is a chronic inflammatory skin disease characterized by pruritis, cutaneous reactivity and erythma, frequently seen in patients with a history of respiratory allergy (Bird and Montana, 1996). Although the etiology is unknown, the disease clearly has an underlying immunological/inflammatory basis with mast cells, Th-2 cells and eosinophils being implicated (Bird and Montana 1996). Furthermore, inflammatory cytokines, particularly IL-4 and TNF-α, are thought to be important factors in the etiology of the progression of the disease and are thus potential targets for drug intervention (Bird and Montana, 1996). Some (Grewe et al., 1982; Townley, 1993), but not all (Gantner et al., 1997c), reports indicate that in monocytes from atopic individuals, cyclic AMP PDE activity is elevated although the basis for this is uncertain (Giembycz and Souness, 1995). Reduced β-adrenoceptor responsiveness (Grewe et al., 1982), as well as enhanced release of histamine, IL-4, PGE_2 and IgE have been linked to the increased cyclic AMP PDE in MNL from atopic individuals (Butler et al., 1983; Cooper et al., 1985; Chan et al., 1993a,b).

10.1. Preclinical models

PDE4 inhibitors reduce eosinophil accumulation in antigen-induced skin inflammation models (Teixeira et al., 1997; Cooper et al., 1999). In a cutaneous inflammation model in guinea-pigs, PDE4 inhibitors suppress the trafficking of antigen [^{111}I]-labelled eosinophils into sensitized skin sites (Cooper et al., 1999). Suppression of PAF and opsonized zymosan-induced skin eosinophilia is also observed (Cooper et al., 1999). A recent, preliminary report shows PDE4 inhibitors to suppress antigen-, but not histamine-induced edema in the skin of sensitized guinea-pigs (Newbold et al., 1999). PDE4 inhibitors reduce passive cutaneous anaphylaxis (PCA) in the skin of rats, mice and guinea-pigs possibly by inhibiting mediator release (Davies and Evans, 1973). Direct evidence for inhibition of mediator release by PDE4 inhibitors has been demonstrated in other skin inflammation models (Griswold et al., 1993).

10.2. Results from clinical studies

Ro 20–1724 and CP-80633 demonstrate some efficacy when applied topically to AD patients (Hanifin, 1991; Hanifin et al., 1996). For example, atizoram, when applied as a topical ointment (0.5%) over 28 days to affected areas in 20 AD patients, demonstrated efficacy with significant reductions in all inflammatory parameters measured (Hanifin et al., 1996).

11. Potential of PDE4 inhibitors in other inflammatory diseases

A glance through the numerous patents on PDE4 inhibitors reveals a large number of indications for which this class of compounds is claimed to be of therapeutic benefit. Many of these are diseases in which TNF-α is implicated as an important pathological factor. In general, there is little hard, published data in experimental animal models to support most of these claims. However, several studies in animal models have demonstrated that PDE4 inhibitors suppress inflammation in a variety of tissues other than those mentioned above, which would point to an extended potential therapeutic utility for this class of compounds. Protection against T-cell-mediated liver damage by rolipram and the PDE3 inhibitor, motapizone, may have therapeutic implications for treatment of liver failure caused by viral infections (Gantner et al., 1997a; Xiang et al., 1999). Normalization of very long chain fatty acids and inhibition of inflammatory cytokines by agents that elevate cyclic AMP, including PDE4 inhibitors, may indicate potential in the treatment of X-adrenoleukodystrophy (Pahan et al., 1998). The importance of TNF-α in septic shock has been demonstrated with antibodies in animal and clinical studies (Bloxham, 1994) and PDE4 inhibitors lower serum TNF-α and decrease mortality in LPS-induced endotoxic shock in mice (Sekut et al., 1995). Finally, rolipram prevents autoimmune insulitis and diabetes in NOD mice, a disease model in which IFN-γ and IL-12 have been implicated as pathological factors (Liang et al., 1998). These data suggest that PDE4 inhibitors may be useful in the treatment of autoimmune diabetes and other conditions characterized by excessive production of cytokines.

12. Side-effects and strategies to improve therapeutic ratios

From the previous discussion, it is clear that PDE4 inhibitors have considerable therapeutic potential. Furthermore, they are generally devoid of the cardiovascular side-effects (increased cardiac contractility, vasodilatation, potential arrhythmogenic activity) associated with PDE3 inhibitors (Treese and Rhein, 1990). However, they do exhibit a number of side-effects which may limit their potential therapeutic utility, especially if they are to be administered by mouth. This, perhaps, is not surprising in view of the wide-ranging tissue distribution of PDE4.

Gastrointestinal (GI) side-effects have been observed with several PDE4 inhibitors in the clinic. Nausea and vomiting have been reported following administration of rolipram (Eban and Ruther, 1985), tibenalast (Israel et al., 1988), ibudilast (Kawasaki et al., 1992), BRL-61063 (Everitt et al., 1994), zardaverine (even by the inhalation route) (Brunnee et al., 1992), Ariflo (Brown, 1999) and piclamilast (De-Brito et al., 1997) to human subjects. Emesis and

central nervous system side-effects are also observed in dogs and ferrets following administration of PDE4 inhibitors (Heaslip and Evans, 1995; Duplantier et al., 1996; Robichaud et al., 1999). The mechanism(s) by which PDE4 inhibitors induce these side-effects is/are uncertain but central actions are likely to be involved (Carpenter et al., 1988; Robichaud et al., 1999). PDE4 inhibitors are also potent stimulators of acid secretion (Puurunen et al., 1978) from gastric parietal cells, and this may contribute to the gastrointestinal disturbances which have been reported in the clinic. Other side-effects observed with PDE4 inhibitors, such as the neckache and backache observed with piclamilast (DeBrito et al., 1997), may be structure- rather than mechanism-related since they are not widely reported phenomena with PDE4 inhibitors in the clinic.

Considerable effort is currently being directed towards the improvement of both the efficacy and side-effect profiles (in particular, reduced nausea and vomiting) of this class of compound. Several pharmaceutical companies have attempted to identify compounds that selectively discriminate among the four different PDE4 subtypes (Muller et al., 1996) and it is likely that in vitro and in vivo pharmacological information on such inhibitors will be released in the near future. However, a clear rational/linking therapeutic efficacy coupled with reduced side-effects to selective inhibition of one PDE4 subtype has not so far emerged.

Some adverse effects of PDE inhibitors, notably emesis and acid secretion, appear not to be correlated with inhibition of the catalytic site of PDE4 but are more closely associated with displacement of rolipram from its high-affinity binding site which, as discussed previously, probably represents a conformation of one or more PDE4 subtypes with which rolipram binds with high affinity (HA-PDE4) (Souness and Rao, 1997; Torphy, 1998). Certain anti-inflammatory actions of PDE inhibitors, however, correlate more closely with inhibition of a PDE4 form against which rolipram exhibits relatively low potency (> 100 nM) (LA-PDE4) (Souness and Rao, 1997; Torphy, 1998) and several groups have attempted to identify compounds which potently and preferentially inhibit at this site with a view to reducing side-effects. Although compounds which show preference for the low-affinity PDE4 have been synthesized (Cheng et al., 1995; Masamune et al., 1995; Duplantier et al., 1996), data on their efficacy and side-effects profile have not been forthcoming. It is probable that compounds with the requisite LA-PDE4/HA-PDE4 ratio, as well as appropriate physicochemical properties required of an orally bioavailable drug, have not been identified, thereby frustrating attempts to determine whether this is a viable approach to improve the therapeutic ratio. The view, that the anti-inflammatory actions of PDE4 inhibitors are linked to LA-PDE4 whereas the side-effects are associated with HA-PDE4, is almost certainly an oversimplification. The multitude of PDE4 forms, their complex cell/tissue distribution, and as their differential up- and downregulation in response to a variety of extracellular (including inflammatory) stimuli, mean that a multitude of factors have to be considered to formulate a rational approach in identifying improved PDE4 inhibitors. If the ideas proposed by Houslay et al. (1998) have substance, it is even possible that different functional responses in individual inflammatory cells are coupled to different cyclic AMP generating, detecting and degrading enzymes in discreet intracellular compartments. Evidence exists that structural features of different PDE4 forms determining subcellular localization also influence sensitivity to inhibitors. The logical consequence is that structure–activity relationships exhibited by a range of PDE4 inhibitors against different functional responses in the same inflammatory cell may not be identical.

13. Conclusions

Ten years have passed since the publication of the first information highlighting the potential of PDE4 inhibitors for the treatment of asthma, and where do we stand? A large number of structurally diverse compounds have been synthesised and, although many show impressive efficacy in preclinical models, results in asthma patients to date have been disappointing. Arofylline, CDP-840 and Ariflo and others that have been evaluated orally and all, with the exception of Ariflo, have exhibited unimpressive efficacy at tolerated doses. Ariflo, itself, appears to be making greater progress as a potential treatment for COPD than for asthma. No greater success has

been achieved by the inhalation route with compounds such as piclamilast, the side-effect of which prohibited raising of doses sufficiently to demonstrate efficacy (DeBrito et al., 1997).

Few studies have been conducted in other diseases. Piclamilast exerts effects on clinical markers in rheumatoid arthritis (Chikanza et al., 1996), but unimpressive efficacy and side-effects issues resulted in Rhône-Poulenc Rorer discontinuing its development. Other companies are reported to be taking their compounds into RA clinical trials, but no information on efficacy has so far been reported. This is also the case for MS where considerable pre-clinical data would suggest that PDE4 inhibitors alone or in combination with other drugs may have a beneficial impact on the disease. There have been reports that PDE4 inhibitors reduce symptoms in patients with atopic dermatitis (Hanifin, 1991; Hanifin et al., 1996) and psoriasis (Sawiski et al., 1979). It is known that Pfizer are currently developing the rolipram analogue, atizoram, for the treatment of atopic dermatitis but there are limited reports updating progress in the development of compounds for dermatological applications.

The most exciting development has been the demonstration of efficacy by Ariflo in phase II/III clinical trials in COPD patients (Brown, 1999). However, COPD is a term that encompasses several fragmented patient populations who manifest, to differing degrees, emphysematous and bronchitic symptoms (Barnes, 1998). Which patient group will benefit the most from Ariflo therapy, and whether combination therapy with steroids and bronchodilators will provide added benefits, have yet to be established. The mechanism(s) by which Ariflo exerts its beneficial effects in COPD remains unknown, although inhibition of TNF-α release, neutrophil recruitment and activation, epithelial cytoprotection, suppression or excitatory nerve-induced inflammation and bronchodilatation are potential candidates (Torphy, 1998).

As mentioned previously, it is possible that dose limitations imposed by the onset of side-effects currently prevent the full potential of PDE4 inhibitors being realised, particularly in the treatment of asthma. In spite of numerous setbacks, many companies remain active in the field and are testing novel PDE4 inhibitors in the clinic, although it is uncertain what advantage these hold over those that have already been evaluated. The therapeutic ratios of all the compounds that have entered clinical trials at the time of writing are either non-existent or slim. Even Ariflo, which has been tested at 15 mg (b.i.d.) in COPD patients, is reported to induce nausea and vomiting in asthma patients at 15 and 20 mg (Brown, 1999). As discussed above, rational or semi-rational approaches to improve therapeutic ratios, based on our vastly improved knowledge of the molecular biology and biochemistry of PDE4, have not yet been fruitful. Whatever the difficulties, PDE4 remains a highly active field of drug research in which there is justifiable optimism for a successful commercial outcome.

References

Adams, D.O., Hamilton, T.A., 1984. The cell biology of macrophage activation. Annu. Rev. Immunol. 2, 283–318.

Adamson, R.H., Liu, B., Fry, G.N., Rubin, L.L., Curry, F.E., 1998. Microvascular permeability and number of tight junctions are modulated by cAMP. Am. J. Physiol. 274, H1885–H1894.

Alvarez, R., Sette, C., Yang, D., Eglen, R.M., Wilhelm, R., Shelton, E.R., Conti, M., 1995. Activation and selective inhibition of a cyclic AMP-specific phosphodiesterase, PDE4D3. Mol. Pharmacol. 48, 616–622.

Alves, A.C., Pires, A.L., Cruz, H.N., Serra, M.F., Diaz, B.L., Cordeiro, R.S., Lagente, V., Martins, M.A., 1996. Selective inhibition of phosphodiesterase type IV suppresses the chemotactic responsiveness of rat eosinophils in vitro. Eur. J. Pharmacol. 312, 89–96.

Anderson, R., Mahomed, A., Goolam, T.A.J., Ramafi, G., Feldman, C., 1998. Effect of rolipram and dibutyryl cyclic AMP on resequestration of cytosolic calcium in FMLP-activated human neutrophils. Br. J. Pharmacol. 124, 547–555.

Aoshiba, K., Nagai, A., Konno, K., 1995. Erythromycin shortens neutrophil survival by accelerating apoptosis. Antimicrob. Agents Chemother. 39, 872–877.

Au, B.T., Teixeira, M.M., Collins, P.D., Williams, T.J., 1998. Effect of PDE4 inhibitors on zymosan-induced IL-8 release from human neutrophils: synergism with prostanoids and salbutamol. Br. J. Pharmacol. 123, 1260–1266.

Averill, L.E., Stein, R.L., Kammer, G.M., 1988. Control of human T-lymphocyte interleukin-2 production by a cyclic AMP-dependent pathway. Cell. Immunol. 115, 88–99.

Badger, A.M., Lee, J.C., 1997. Advances in antiarthritic therapeutics. Drug Discovery Today 2, 427–435.

Badwey, J.A., Karnovsky, M.L., 1980. Active oxygen species and the functions of phagocytic leukocytes. Annu. Rev. Biochem. 49, 695–726.

Ballestas, M.E., Benveniste, E.N., 1997. Elevation of cyclic AMP

levels in astrocytes antagonizes cytokine-induced adhesion molecule expression. J. Neurochem. 69, 1438–1448.

Barnes, P.J., 1998. New therapies for chronic obstructive pulmonary disease. Thorax 53, 137–147.

Barnes, P.J., Chung, K.F., Page, C.P., 1988. Inflammatory mediators and asthma. Pharmacol. Rev. 40, 49–84.

Barnette, M.S., Christensen, S.B., Essayan, D.M., Grous, M., Probhakar, U., Rush, J.A., Kagey-Sobotka, A., Torphy, T.J., 1998. SB 207499 (Ariflo), a potent and selective second-generation phosphodiesterase 4 inhibitor: in vitro anti-inflammatory actions. J. Pharmacol. Exp. Ther. 284, 420–426.

Barnette, M.S., Christensen, S.B., Underwood, D.C., Torphy, T.J., 1996a. Phosphodiesterase 4: biological underpinnings for the design of improved inhibitors. Pharmacol. Commun. 8, 65–73.

Barnette, M.S., Grous, M., Cieslinski, L.B., Burman, M., Christensen, S.B., Torphy, T.J., 1995a. Inhibitors of phosphodiesterase IV (PDE IV) increase acid secretion in rabbit isolated gastric glands: correlation between function and interaction with a high-affinity rolipram binding site. J. Pharmacol. Exp. Ther. 273, 1396–1402.

Barnette, M.S., Manning, C.D., Cieslinski, L.B., Burman, M., Christensen, S.B., Torphy, T.J., 1995b. The ability of phosphodiesterase IV inhibitors to suppress superoxide production in guinea pig eosinophils is correlated with inhibition of phosphodiesterase IV catalytic activity. J. Pharmacol. Exp. Ther. 273, 674–679.

Barnette, M.S., O'Leary Bartus, J., Burman, M., Christensen, S.B., Cieslinski, L.B., Esser, K.B., Probhakar, U.S., Rush, J.A., Torphy, T.J., 1996b. Association of the anti-inflammatory activity of phosphodiesterase 4 (PDE4) inhibitors with either inhibition of PDE4 catalytic activity or competition for [^{3}H] rolipram binding. Biochem. Pharmacol. 51, 949–956.

Baroja, M.L., Cieslinski, L.B., Torphy, T.J., Wange, R.L., Madrenas, J., 1999. Specific CD3ε association of a phosphodiesterase 4B isoform determines its selective tyrosine phosphorylation after CD3 ligation. J. Immunol. 162, 2016–2023.

Beavo, J.A., Conti, M., Heaslip, R.J., 1994. Multi-cyclic nucleotide phosphodiesterases. Mol. Pharmacol. 46, 399–405.

Belayev, L., Busto, R., Ikeda, M., Rubin, L.L., Kajiwara, A., Morgan, L., Ginsberg, M.D., 1998. Protection against blood–brain barrier disruption in focal cerebral ischemia by the type IV phosphodiesterase inhibitor BBB022: a quantitative study. Brain Res. 787, 277–285.

Berends, C., Dijkhuizen, B., De-Monchy, J.G.R., Dubois, A.E.J., Gerritsen, J., Kauffman, H.F., 1997. Inhibition of PAF-induced expression of CD11b and shedding of L-selectin on human neutrophils and eosinophils by the type IV selective PDE inhibitor, rolipram. Eur. Respir. J. 10, 1000–1007.

Bessler, H., Gilgal, R., Djaldatti, R., Zahavi, M., 1986. Effect of pentoxifylline on the phagocytic activity, cyclic AMP levels, and superoxide anion production by monocytes and polymorphonuclear cells. J. Leukocyte Biol. 40, 747–754.

Betz, M., Fox, B.S., 1991. Prostaglandin E_2 inhibits production of Th-1 lymphokines but not of Th-2 lymphokines. J. Immunol. 146, 108–113.

Bird, J., Montana, J., 1996. New therapeutic approaches to atopic dermatitis. Exp. Opin. Invest. Drugs 5, 1173–1180.

Blease, K., Burkegaffney, A., Hellewell, P.G., 1998. Modulation of cell adhesion molecule expression and function on human lung microvascular endothelial cells by inhibition of phosphodiesterases 3 and 4. Br. J. Pharmacol. 124, 229–237.

Block, F., Tondar, A., Schmidt, W., Schwarz, M., 1997. Delayed treatment with rolipram protects against neuronal damage following global ischemia in rats. NeuroReport 8, 3829–3832.

Bloxham, D.P., 1994. Therapeutic applications of anti-TNF monoclonal antibodies. Exp. Opin. Invest. Drugs 3, 907–912.

Bochner, B.S., Undem, B.J., Lichtenstein, L.M., 1994. Immunological aspects of allergic asthma. Annu. Rev. Immunol. 12, 295–335.

Bonta, I.L., Parnham, M.J., Van Vliet, L., 1978. Combination of theophylline and prostaglandin E1 as inhibitors of the adjuvant-induced arthritis syndrome of rats. Ann. Rheum. Dis. 37, 212–217.

Brown, W.M., 1999. SB-207499 SmithKline Beecham. Curr. Opin. Anti-Inflammatory Immunomodulatory Invest. Drugs 1, 39–47.

Brunnee, T., Engelstatter, R., Steinijans, V.W., Kunkel, G., 1992. Bronchodilatory effect of zardaverine, a phosphodiesterases III and IV inhibitor, in patients with asthma. Eur. Respir. J. 5, 982–985.

Butcher, R.W., Sutherland, E.W., 1962. Adenosine 3',5'-phosphate in biological materials. J. Biol. Chem. 237, 1244–1250.

Butler, J.M., Chan, S.C., Stevens, S., Hanifin, J.M., 1983. Increased leukocyte histamine release with elevated cyclic AMP-phosphodiesterase activity in atopic dermatitis. J. Allergy Clin. Immunol. 71, 490–497.

Buttini, M., Mir, A., Appel, K., Wiederhold, K.H., Limonta, S., Gebickeharter, P.J., Boddeke, H.W.G.M., 1997. Lipopolysaccharide induces expression of tumour necrosis factor-alpha in rat brain: inhibition by methylprednisolone and by rolipram. Br. J. Pharmacol. 122, 1483–1489.

Carpenter, D.O., Briggs, D.B., Knox, A.P., Strominger, N., 1988. Excitation of area postrema neurons by transmitters, peptides, and cyclic nucleotides. J. Neurophys. 59, 358–369.

Chan, A.C., Desai, D.M., Weiss, A., 1994. The role of protein tyrosine kinases and protein tyrosine phosphatases in T-cell antigen receptor signal transduction. Annu. Rev. Immunol. 12, 555–592.

Chan, S.C., Kim, J.-W., Henderson, W.R., Hanifin, J.M., 1993a. Altered prostaglandin E_2 regulation of cytokine production in atopic dermatitis. J. Immunol. 151, 3345–3352.

Chan, S.C., Li, S.-H., Hanifin, J.M., 1993b. Increased interleukin-4 production by atopic mononuclear leukocytes correlates with increased cyclic adenosine monophosphate-phosphodiesterase activity and is reversible by phosphodiesterase inhibition. J. Invest. Dermatol. 100, 681–684.

Chen, D., Rothenberg, E.V., 1994. Interleukin-2 transcription factors as molecular targets of cAMP inhibition: delayed inhibition kinetics and combinatorial transcription roles. J. Exp. Med. 179, 931–942.

Cheng, J.B., Cooper, K., Duplantier, A.J., Eggler, J.F., Kraus, K.G., Marshall, S.C., Marfat, A., Masamune, H., Shirley, J.T., Tickner, J.E., Umland, J.P., 1995. Synthesis and in vitro profile of a novel series of catecholbezimidazoles. The discov-

ery of potent, selective phosphodiesterase type IV inhibitors with greatly attenuated affinity for the [^{3}H] rolipram binding site. Bioorg. Med. Chem. Lett. 5, 1969–1972.

Cher, D.J., Mosmann, T.R., 1987. Two types of murine helper T-cell clone: II. Delayed type hypersensitivity is mediated by Th-1 clones. J. Immunol. 138, 3688–3694.

Chikanza, I.C., Jawed, S.J., Blake, D.R., Perrot, S., Menkes, C.J., Barnes, C.G., Perry, J.D., Wright, M.G., 1996. Treatment of patients with rheumatoid arthritis with RP 73401 phosphodiesterase type IV inhibitor. Arthritis Rheum. 39, S282.

Chini, C.C.S., Grande, J.P., Chini, E.N., Dousa, T.P., 1997. Compartmentalization of cAMP signaling in mesangial cells by phosphodiesterase isozymes PDE3 and PDE4. Regulation of superoxidation and mitogenesis. J. Biol. Chem. 272, 9854–9859.

Christensen, S.B., DeWolf, W.E. Jr., Ryan, M.D., Torphy, T.J., 1996. Molecular aspects of inhibitor interaction with PDE4. In: Dent, G., Rabe, K., Schudt, C. (Eds.), Handbook of Immunopharmacology: Phosphodiesterase Inhibitors. Academic Press, London, pp. 185–207.

Cohan, V.L., Johnson, K.L., Breslow, R., Cheng, J.B., Showell, H.J., 1992. PDE IV is the predominant PDE isoenzyme regulating chemotactic factor-mediated guinea-pig eosinophil functions in vitro. J. Allergy Clin. Immunol. 89, 663.

Compton, C.H., Cedar, E., Nieman, R.B., Amit, O., Langley, S.J., Sapene, M., 1999a. Ariflo (SB 207499) improves pulmonary function in patients with asthma: results of a study in patients taking inhaled corticosteroids. Am. J. Respir. Crit Care Med. 159, A624a.

Compton, C.H., Gubb, J., Cedar, E., Bakst, A., Nieman, R.B., Amit, O., Ayres, J., Brambilla, C., 1999b. Ariflo (SB 207499), a second-generation, oral PDE4 inhibitor, improves quality of life in patients with COPD. Am. J. Respir. Crit Care Med. 159, A522c.

Compton, C.H., Gubb, J., Cedar, E., Nieman, R.B., Amit, O., Brambilla, C., Ayres, J., 1999c. The efficacy of Ariflo (SB 207499), a second-generation, oral PDE4 inhibitor, in patients with COPD. Am. J. Respir. Crit Care Med. 159, A806b.

Conti, M., Iona, S., Cuomo, M., Swinnen, J.V., Odeh, J., Svoboda, M.E., 1995. Characterization of a hormone-inducible, high-affinity adenosine 3′–5′-cyclic monophosphate phosphodiesterase from rat Sertoli cell. Biochemistry 34, 7979–7987.

Conti, M., Swinnen, J.V., 1990. Structure and function of the rolipram-sensitive, low K_m cyclic AMP phosphodiesterase: a family of highly related enzymes. In: Houslay, M.D., Beavo, J.A (Eds.), Molecular Pharmacology of Cell Regulation: Cyclic Nucleotide Phosphodiesterase Structure, Regulation and Drug Action. Wiley, New York, pp. 243–266.

Cooper, K.D., Kang, K., Chan, S.C., Hanifin, J.M., 1985. Phosphodiesterase inhibition by Ro 20–1724 reduces hyper-IgE synthesis by atopic dermatitis cells in vitro. J. Invest. Dermatol. 84, 477–482.

Cooper, N., Teixeira, M.M., Warneck, J., Miotla, J.M., Wills, R.E., Macari, D.M.T., Gristwood, R.W., Hellewell, P.G., 1999. A comparison of the inhibitory activity of PDE4 inhibitors on leukocyte PDE4 activity in vitro and eosinophil trafficking in vivo. Br. J. Pharmacol. 126, 1863–1871.

Coqueret, O., Boichot, E., Lagente, V., 1997. Selective type IV phosphodiesterase inhibitors prevent IL-4-induced IgE production by human peripheral blood mononuclear cells. Clin. Exp. Allergy 27, 816–823.

Crocker, I.C., Townley, R.G., Khan, M.M., 1994. Phosphodiesterase inhibitors modulate cytokine secretion and proliferation. J. Allergy Clin. Immunol. 93, 286.

Crummey, A., Harper, G.P., Boyle, E.A., Mangan, F.R., 1987. Inhibition of arachidonic acid-induced ear edema as a model for assessing topical anti-inflammatory compounds. Agents Actions 20, 69–76.

Danahay, H., Broadley, K.J., 1997. Effects of inhibitors of phosphodiesterase, on antigen-induced bronchial hyperreactivity in conscious sensitized guinea-pigs and airway leukocyte infiltration. Br. J. Pharmacol. 120, 289–297.

Danahay, H., Broadley, K.J., 1998. PDE4 inhibition and a corticosteroid in chronically antigen exposed conscious guinea-pigs. Clin. Exp. Allergy 28, 513–522.

Davies, G.E., Evans, D.P., 1973. Studies with two new phosphodiesterase inhibitors (ICI 58,301 and ICI 63,197) on anaphylaxis in guinea-pigs, mice and rats. Int. Arch. Allergy Appl. Immunol. 45, 467–478.

DeBrito, F.B., Souness, J.E., Warne, P.J., 1997. Type 4 phosphodiesterase inhibitors and their potential in the treatment of inflammatory disease. Emerging Drugs 2, 249–268.

Del Prete, G., Maggi, E., Parronchi, P., Chretien, I., Tiri, A., Macchia, D., Ricci, M., Banchereau, J., De Vries, J., Romagnani, S., 1988. IL-4 is an essential factor for the IgE synthesis induced in vitro by human T-cell clones and their supernatants. J. Immunol. 140, 4193–4198.

De Moraes, V.L.G., Singer, M., Vargaftig, B.B., Chignard, M., 1998. Effects of rolipram on cyclic AMP levels in alveolar macrophages and lipopolysaccharide-induced inflammation in mouse lung. Br. J. Pharmacol. 123, 631–636.

Denis, D., Riendeau, D., 1999. Phosphodiesterase 4-dependent regulation of cyclic AMP levels and leukotriene B4 biosynthesis in human polymorphonuclear leukocytes. Eur. J. Pharmacol. 367, 343–350.

Dent, G., Giembycz, M.A., Evans, P.M., Rabe, K.F.; Barnes, P.J., 1994. Suppression of human eosinophil respiratory burst and cyclic AMP hydrolysis by inhibitors of type IV phosphodiesterase: interaction with the beta-adrenoceptor agonist, albuterol. J. Pharmacol. Exp. Ther. 271, 1167–1174.

Dent, G., Giembycz, M.A., Rabe, K.F., Barnes, P.J., 1991. Inhibition of eosinophil cyclic nucleotide PDE activity and opsonised zymosan-induced respiratory burst by 'type IV'-selective PDE inhibitors. Br. J. Pharmacol. 103, 1339–1346.

Dent, G., White, S.R., Tenor, H., Bodtke, K., Schudt, C., Leff, A.R., Magnussen, H., Rabe, K.F., 1998. Cyclic nucleotide phosphodiesterase in human bronchial epithelial cells: characterization of isoenzymes and functional effects of PDE inhibitors. Pulm. Pharmacol. Ther. 11, 47–56.

Derian, C.K., Santullim, R.J., Rao, P.E., Solomon, H.F., Barrett, J.A., 1995. Inhibition of chemotactic peptide-induced neutrophil adhesion to vascular endothelium by cAMP modulators. J. Immunol. 154, 308–317.

Dinter, H., Onuffer, J., Faulds, D., Perez, H.D., 1997. Phosphodi-

esterase inhibitors in the treatment of multiple sclerosis. J. Mol. Med. 75, 95–102.

DiSanto, M.E., Glaser, K.B., Heaslip, R.J., 1995. Phospholipid regulation of a cyclic AMP-specific phosphodiesterase (PDE4) from U937 cells. Cell. Signalling 7, 827–835.

DiSanto, M.E., Heaslip, R.J., 1995. Rolipram inhibition of phosphodiesterase-4 activation. Eur. J. Pharmacol. 290, 169–172.

Dowling, R.B., Johnson, M., Cole, P.J., Wilson, R., 1997. The effect of rolipram, a type IV phosphodiesterase inhibitor, on *Pseudomonas aeruginosa* infection of respiratory mucosa. J. Pharmacol. Exp. Ther. 282, 1565–1571.

Duplantier, A.J., Biggers, M.S., Chambers, R.J., Cheng, J.B., Cooper, K., Damon, D.B., Eggler, J.F., Kraus, K.G., Marfat, A., Masamune, H., Pillar, J.S., Shirley, J.T., Umland, J.P., Watson, J.W., 1996. Biarylcarboxylic acids and -amides: Inhibition of phosphodiesterase type IV versus [^{3}H] rolipram binding activity and their relationship to emetic behavior in the ferret. J. Med. Chem. 39, 120–125.

Eban, E., Ruther, E., 1985. Animal trial prediction and clinical effect of new chemical substances to be used as antipsychotic agents. Pharmacopsychiatry 18, 69–70.

Ekholm, D., Hemmer, B., Gao, G., Vergelli, M., Martin, R., Manganiello, V., 1997. Differential expression of cyclic nucleotide phosphodiesterases 3 and 4 activities in human T-cell clones specific for myelin basic protein. J. Immunol. 159, 1520–1529.

Elbawab, S., Macovschi, O., Sette, C., Conti, M., Lagarde, M., Nemoz, G., Prigent, A.F., 1997. Selective stimulation of a cAMP-specific phosphodiesterase (PDE4A5) isoform by phosphatidic acid molecular species endogenously formed in rat thymocytes. Eur. J. Biochem. 247, 1151–1157.

Engels, P., Fichtel, K., Lubbert, H., 1994. Expression and regulation of human and rat phosphodiesterase type IV isogenes. FEBS Lett. 350, 291–295.

Epstein, P.M., Hachisu, R., 1984. Cyclic nucleotide phosphodiesterase in normal and leukemic human lymphocytes and lymphoblasts. Adv. Cyclic Nucl. Protein Phosphorylation Res. 16, 303–324.

Erdogan, S., Houslay, M.D., 1997. Challenge of human Jurkat T-cells with the adenylate cyclase activator forskolin elicits major changes in cAMP phosphodiesterase (PDE) expression by upregulating PDE3 and inducing PDE4D1 and PDE4D2 splice variants as well as downregulating a novel PDE4A splice variant. Biochem. J. 321, 165–175.

Escofier, N., Boichot, E., Germain, N., Silva, P.M.R.E., Martins, M.A., Lagente, V., 1999. Effects of interleukin-10 and modulators of cyclic AMP formation on endotoxin-induced inflammation in rat lung. Fundam. Clin. Pharmacol. 1, 96–101.

Essayan, D.M., Huang, S.-K., Kagey-Sabotka, A., Lichtenstein, L.M., 1995. Effects of nonselective and isozyme-selective cyclic nucleotide phosphodiesterase inhibitors on antigen-induced cytokine gene expression in peripheral blood mononuclear cells. Am. J. Respir. Cell. Mol. Biol. 13, 692–702.

Essayan, D.M., Huang, S.K., Kabey-Sobatka, A., Lichtenstein, L.M., 1997a. Differential efficacy of lymphocyte- and monocyte-selective pretreatment with a type 4 phosphodiesterase inhibitor on antigen-driven proliferation and cytokine gene expression. J. Allergy Clin. Immunol. 99, 28–37.

Essayan, D.M., Huang, S.-K., Undem, B.J., 1994. Antigen- and mitogen-induced proliferative responses of peripheral blood mononuclear cells by non-selective and isozyme-selective cyclic nucleotide phosphodiesterase inhibitors. J. Immunol. 153, 3408–3416.

Essayan, D.M., Kagey-Sabotka, A., Lichtenstein, L.M., Huang, S.K., 1997b. Regulation of interleukin-13 by type 4 cyclic nucleotide phosphodiesterase (PDE) inhibitors in allergen-specific T-lymphocyte clones. Biochem. Pharmacol. 53, 1055–1060.

Everitt, D.E., Boike, S., Davies, B., Zariffa, N., Audet, P., Brown, L., Freed, M., Ilson, B., Esser, K., O'Leary-Bartus, J., Slack, G., Smith, G., Jordasky, D., 1994. Pharmacokinetics, tolerability and TNF inhibitory activity of 1,3-di(cyclopropyl-methyl)-8-aminoxanthine (BRL 61063). Clin. Pharmacol. Ther. 55, 150.

Ezeamuzie, C.I., Alhage, M., 1998. Effects of some anti-asthma drugs on human eosinophil superoxide anions release and degranulation. Int. Arch. Allergy Immunol. 115, 162–168.

Faux, M.C., Scott, J.D., 1996. Molecular glue: kinase anchoring and scaffolding proteins. Cell 85, 9–12.

Feuerstein, G.Z., Neville, L.F., Rabinovici, R., 1995. Pulmonary TNF-α is a critical mediator in adult respiratory distress syndrome. J. Endotoxin Res. 2, 189–193.

Fischer, D.A., Smith, J.F., Pillar, J.S., St. Denis, S.H., Cheng, J.B., 1998a. Isolation and characterization of PDE8A, a novel human cAMP-specific phosphodiesterase. Biochem. Biophys. Res. Commun. 246, 570–577.

Fischer, D.A., Smith, J.F., Pillar, J.S., St. Denis, S.H., Cheng, J.B., 1998b. Isolation and characterization of PDE9A, a novel human cGMP-specific phosphodiesterase. J. Biol. Chem. 273, 15559–15564.

Fonteh, A.N., Winkler, J.D., Torphy, T.J., Heravi, J., Undem, B.J., Chilton, F.H., 1993. Influence of isoproterenol and phosphodiesterase inhibitors on platelet-activating factor biosynthesis in the human neutrophil. J. Immunol. 151, 339–350.

Foster, R.W., Rakshi, K., Carpenter, J.R., Small, R.C., 1992. Trials of the bronchodilator activity of the isoenzyme-selective phosphodiesterase inhibitor, AH 21-132, in healthy volunteers during a methacholine challenge. Br. J. Clin. Pharmacol. 34, 527–534.

Francis, S.H., Colbran, J.L., McAllister Lucas, L.M., Corbin, J.D., 1994. Zinc interactions and conserved motifs of the cGMP-binding cGMP-specific phosphodiesterase suggest that it is a zinc hydrolase. J. Biol. Chem. 269, 22477–22480.

Frossard, N., Landry, Y., Pauli, G., Ruckstuhl, M., 1981. Effects of cyclic AMP- and cyclic GMP-phosphodiesterase inhibitors on immunological release of histamine and on lung contraction. Br. J. Pharmacol. 73, 933–938.

Fuhrmann, M., Jahn, H.U., Seybold, J., Neurohr, C., Barnes, P.J., Hippenstiel, S., Kraemer, H.J., Suttorp, N., 1999. Identification and function of cyclic nucleotide phosphodiesterase isoenzymes in airway epithelial cells. Am. J. Respir. Cell. Mol. Biol. 20, 292–302.

Fujimoto, T., Sakoda, S., Fujimura, H., Yanagihara, T., 1999. Ibudilast, a phosphodiesterase inhibitor, ameliorates experimental autoimmune encephalomyelitis in Dark August rats. J. Neuroimmunol. 95, 35–42.

Gantner, F., Gotz, C., Gekeler, V., Schudt, C., Wendel, A., Hatzelmann, A., 1998. Phosphodiesterase profile of human B-lymphocytes from normal and atopic donors and the effects of PDE inhibition on B-cell proliferation. Br. J. Pharmacol. 123, 1031–1038.

Gantner, F., Kupferschmidt, R., Schudt, C., Wendel, A., Hatzelmann, A., 1997a. In vitro differentiation of human monocytes to macrophages: change of PDE profile and its relationship to suppression of tumour necrosis factor-alpha release by PDE inhibitors. Br. J. Pharmacol. 121, 221–231.

Gantner, F., Kusters, S., Wendel, A., Hatzelmann, A., Schudt, C., Tiegs, G., 1997b. Protection from T-cell-mediated murine liver failure by phosphodiesterase inhibitors. J. Pharmacol. Exp. Ther. 280, 53–60.

Gantner, F., Tenor, H., Gekeler, V., Schudt, C., Wendel, A., Hatzelmann, A., 1997c. Phosphodiesterase profiles of highly purified human peripheral blood leukocyte populations from normal and atopic individuals: a comparative study. J. Allergy Clin. Immunol. 100, 527–535.

Genain, C.P., Roberts, T., Davis, R.L., Nguyen, M.-H., Uccelli, A., Faulds, D., Li, Y., Hedgpeth, J., Hauser, S.L., 1995. Prevention of autoimmune demyelination in non-human primates by a cAMP-specific phosphodiesterase inhibitor. Proc. Natl. Acad. Sci. U. S. A. 92, 3601–3605.

Giembycz, M.A., 1996. Phosphodiesterase 4 and tolerance to β_2-adrenoceptor agonists in asthma. Trends Pharmacol. Sci. 17, 331–336.

Giembycz, M.A., Corrigan, C.J., Seybold, J., Newton, R., Barnes, P.J., 1996. Identification of cyclic AMP phosphodiesterases 3, 4 and 7 in human $CD4^+$ and $CD8^+$ T-lymphocytes: role in regulating proliferation and biosynthesis of interleukin-2. Br. J. Pharmacol. 118, 1945–1958.

Giembycz, M.A., Souness, J.E., 1995. Phosphodiesterase IV inhibitors as potential therapeutic agents in allergic disease. In: Townley, R.G., Agarwal, D.K. (Eds.), Immunopharmacology of Allergic Disease. Marcel Dekker, New York, pp. 523–560.

Godfrey, R.W., Manzi, R.M., Gennaro, D.E., Hoffstein, S.T., 1987. Phospholipid and arachidonic acid metabolism in zymosan-stimulated human monocytes: modulation by cyclic AMP. J. Cell. Physiol. 131, 384–392.

Gordon, J.R., Burd, P.R., Galli, S.J., 1990. Mast cells as a source of multifunctional cytokines. Immunol. Today 11, 458–464.

Gordon, T., Frennette, R., Ducharme, Y., Pon, D., 1999. Anti-inflammatory effects of a PDE4 inhibitor in a rat model of chronic bronchitis. Am. J. Respir. Crit. Care Med. 159, A89.

Gozzard, N., El-Hashim, A., Herd, C.M., Blake, S.M., Holbrook, M., Hughes, B., Higg, G.A., Page, C.P., 1996. Effect of the glucocorticoid budesonide and a novel phosphodiesterase type 4 inhibitor CDP-840 on antigen-induced airway responses in neonatally immunised rabbits. Br. J. Pharmacol. 118, 1201–1208.

Greten, T.F., Sinha, B., Haslberger, C., Eigler, A., Endres, S., 1996. Cicaprost and the type IV phosphodiesterase inhibitor, rolipram, synergize in suppression of tumour necrosis factor-alpha synthesis. Eur. J. Pharmacol. 299, 229–233.

Grewe, S., Chan, S.C., Hanifin, J.M., 1982. Elevated leukocyte cyclic AMP-phosphodiesterase in atopic disease. J. Allergy Clin. Immunol. 70, 452–457.

Griswold, D.E., Webb, E.F., Badger, A.M., Gorycki, P.D., Levandoski, P.A., Barnette, M.A., Grous, M., Christensen, S., Torphy, T.J., 1998. SB 207499 (Ariflo), a second-generation phosphodiesterase 4 inhibitor, reduces tumor necrosis factor-alpha and interleukin-4 production in vivo. J. Pharmacol. Exp. Ther. 287, 705–711.

Griswold, D.E., Webb, E.F., Breton, J., White, J.R., Marshall, P.J., Torphy, T.J., 1993. Effect of selective phosphodiesterase type IV inhibitor, rolipram, on fluid and cellular phases of inflammatory responses. Inflammation 17, 333–344.

Hanifin, J.M., 1991. Atopic dermatitis: new therapeutic considerations. J. Am. Acad. Dermatol. 24, 1097–1101.

Hanifin, J.M., Butler, J.M., Chan, S.C., 1985. Immunopharmacology of the atopic diseases. J. Invest. Dermatol. 85, 161s–164s.

Hanifin, J.M., Chan, S.C., Cheng, J.B., Tofte, S.J., Henderson, W.R., Kirby, D.S., Weiner, E.S., 1996. Type 4 phosphodiesterase inhibitors have clinical and in vitro anti-inflammatory effects in atopic dermatitis. J. Invest. Dermatol. 107, 51–56.

Harbinson, P.L., MacLeod, D., Hawksworth, R., O'Toole, S., Sullivan, P.J., Heath, P., Kilfeather, S., Page, C.P., Costello, J., Holgate, S.T., Lee, T.H., 1997. The effect of a novel orally active selective PDE4 isoenzyme inhibitor (CDP-840) on allergen-induced responses in asthmatic subjects. Eur. Respir. J. 10, 1008–1014.

Harris, A.L., Connell, M.J., Ferguson, E.W., Wallace, A.M., Gordon, R.J., Pagani, E.D., Silver, P.J., 1989. Role of low K_m cyclic AMP phosphodiesterase inhibition in tracheal relaxation and bronchodilation in the guinea-pig. J. Pharmacol. Exp. Ther. 251, 199–206.

Harvath, L., Robbins, J.D., Russell, A.A., Seaman, K.B., 1991. Cyclic AMP and human neutrophil chemotaxis. Elevation of cyclic AMP differentially affects chemotactic responsiveness. J. Immunol. 146, 224–232.

Hatzelmann, A., Tenor, H., Schudt, C., 1995. Differential effects of non-selective and selective phosphodiesterase inhibitors on human eosinophil functions. Br. J. Pharmacol. 114, 821–831.

Hayashi, M., Matsushima, K., Ohashi, H., Tsunoda, H., Murase, S., Kawarada, Y., Tanaka, T., 1998. Molecular cloning and characterization of human PDE8B, a novel thyroid-specific isoenzyme of 3',5'-cyclic nucleotide phosphodiesterase. Biochem. Biophys. Res. Commun. 250, 751–756.

Heaslip, R.J., Evans, D.Y., 1995. Emetic, central nervous system, and pulmonary activities of rolipram in the dog. Eur. J. Pharmacol. 286, 281–290.

Henderson, B., Pettifer, E., 1989. Arthritogenic actions of recombinant IL-1 and TNF-α in the rabbit. Clin. Exp. Immunol. 75, 306–310.

Hoban, C.J., 1998. Recent advances in preclinical drug development in multiple sclerosis. Exp. Opin. Ther. Patents 8, 831–854.

Holbrook, M., Gozzard, N., James, T., Higgs, G., Hughes, B., 1996. Inhibition of bronchospasm and ozone-induced airway hyperresponsiveness in the guinea-pig by CDP-840, a novel phosphodiesterase type 4 inhibitor. Br. J. Pharmacol. 118, 1192–1200.

Houslay, M.D., Milligan, G., 1997. Tailoring cAMP signalling responses through isoform multiplicity. Trends Biochem. Sci. 22, 217–224.

Houslay, M.D., Sullivan, M., Bolger, G.B., 1998. The multienzyme PDE4 cyclic adenosine monophosphate-specific phosphodiesterase family: intracellular targeting, regulation, and selective inhibition by compounds exerting anti-inflammatory and anti-depressant actions. Adv. Pharmacol. 44, 225–342.

Howell, R.E., Jenkins, L.P., 1994. Inhibition of lipopolysaccharide-induced pulmonary edema by isozyme-selective phosphodiesterase inhibitors in guinea-pigs. Am. J. Respir. Crit. Care Med. 149, A468.

Howell, R.E., Sickles, B.D., Woeppel, S.L., 1993. Pulmonary antiallergic and bronchodilator effects of isozyme-selective phosphodiesterase inhibitors in guinea-pigs. J. Pharmacol. Exp. Ther. 264, 609–615.

Howell, R.E., Woeppel, S.L., Howell, D.E., Rubin, E.B., Jenkins, L.P., Golankiewicz, J.M., Lombardo, L.J., Heaslip, R.J., 1995. Pulmonary antiallergic and anti-inflammatory effects of a novel, orally active phosphodiesterase IV inhibitor (WAY-127093B) in guinea-pigs and rats. Inflammatory Res. 44 (Suppl. 2), S172–S173.

Hsueh, Y.-P., Lai, M.-Z., 1995. c-Jun N-terminal kinase, but not mitogen-activated protein kinase, is sensitive to cAMP inhibition in T-lymphocytes. J. Biol. Chem. 270, 18094–18098.

Hughes, B., Howat, D., Lisle, H., Holbrook, M., James, T., Gozzard, N., Blease, K., Hughes, P., Kingaby, R., Warrellow, G., Alexander, R., Head, J., Boyd, E., Eaton, M., Perry, M., Wales, M., Smith, B., Owens, R., Catterall, C., Lumb, S., Russell, A., Allen, R., Merriman, M., Bloxham, D., Higgs, G., 1996. The inhibition of antigen-induced eosinophilia and bronchoconstriction by CDP-840, a novel stereoselective inhibitor of phosphodiesterase type 4. Br. J. Pharmacol. 118, 1183–1191:

Hulley, P., Hartikka, J., Abdel'Al, S., Engels, P., Buerki, H.R., Weiderhold, K.H., Muller, T., Kelley, P., Lowe, D., Lubbert, H., 1995. Inhibitors of type IV phosphodiesterases reduce the toxicity of MPTP in substantia nigra neurons in vivo. Eur. J. Neurosci. 7, 2431–2440.

Huston, E., Pooley, L., Julien, P., Scotland, G., McPhee, I., Sullivan, M., Bolger, G., Houslay, M., 1997. The human cyclic AMP-specific phosphodiesterase PDE46 (HSPDE4A4B) expressed in transfected COS7 cells occurs as both particulate and cytosolic species which exhibit distinct kinetics of inhibition by the anti-depressant rolipram. J. Biol. Chem. 271, 31334–31344.

Irikura, T., Nishino, K., Hara, S., 1987. Eur. Pat. 215438A2; Chem. Abstr. 107, 183556.

Israel, E., Mathur, P.N., Tachkin, D., Drazen, J.M., 1988. LY 186655 prevents bronchospasm in asthma of moderate severity. Chest 94, 71S.

Jacobitz, S., McLaughlin, M.M., Livi, G.P., Burman, M., Torphy, T.J., 1996. Mapping the functional domains of human recombinant phosphodiesterase 4A: structural requirements for catalytic activity and rolipram binding. Mol. Pharmacol. 50, 891–899.

Jiang, X., Paskind, M., Weltzien, R., Epstein, P.M., 1998. Expression and regulation of mRNA for distinct isoforms of cAMP-specific PDE4 in mitogen-stimulated and leukemic human lymphocytes. Cell Biochem. Biophys. 28, 135–160.

Jin, S.L., Bushnik, T., Lan, L., Conti, M., 1998. Subcellular localization of rolipram-sensitive, cAMP-specific phosphodiesterases. Differential targeting and activation of the splicing variants from the PDE4D gene. J. Biol. Chem. 273, 19672–19678.

Jin, S.L.C., Swinnen, J.V., Conti, M., 1992. Characterization of the structure of a low K_m, rolipram-sensitive cAMP phosphodiesterase. J. Biol. Chem. 267, 18929–18939.

Jones, T.R., McAuliffe, M., McFarlane, C.S., Piechuta, H., MacDonald, D., Rodger, I.W., 1998. Effects of a selective phosphodiesterase IV inhibitor (CDP-840) in a leukotriene-dependent non-human primate model of allergic asthma. Can. J. Physiol. Pharmacol. 76, 210–217.

Jung, E.-Y., Ohshima, Y., Shintaku, N., Sumimoto, S.-I., Heike, T., Katamura, K., Mayumi, M., 1994. Effects of cyclic AMP on expression of LFA-1, Mac-1, and VLA-4 and eosinophilic differentiation of a human leukemic cell line, EoL-1. Eur. J. Hematol. 53, 156–162.

Kambayashi, T., Jacob, C.O., Zhou, D., Mazurek, N., Fong, M., Strassmann, G., 1995. Cyclic nucleotide phosphodiesterase type IV participates in the regulation of IL-10 and the subsequent inhibition of TNF-α and IL-6 release by endotoxin-stimulated macrophages. J. Immunol. 155, 4909–4916.

Kaminuma, O., Kikkawa, H., Matsubara, S., Ikezawa, K., 1996a. Inhibitory effect of a novel phosphodiesterase IV inhibitor, T-440, on antigen- and chemical-mediator-induced bronchoconstrictions in guinea-pigs in vivo. Jpn. J. Pharmacol. 72, 1–8.

Kaminuma, O., Kikkawa, H., Matsubara, S., Ikezawa, K., 1997a. Effect of T-440, a novel type IV phosphodiesterase inhibitor, on allergen-induced immediate and late asthmatic reaction and leukocyte infiltration into the airways of guinea-pigs. Int. Arch. Allergy Immunol. 112, 406–411.

Kaminuma, O., Mori, A., Suko, M., Kikkawa, H., Ikezawa, K., Okudaira, H., 1996b. Interleukin-5 production by peripheral blood mononuclear cells of asthmatic patients is suppressed by T-440: relation to phosphodiesterase inhibition. J. Pharmacol. Exp. Ther. 279, 240–246.

Kaminuma, O., Mori, A., Suko, M., Nishizaki, Y., Kikkawa, H., Ikezawa, K., Okudaira, H., 1996c. A novel phosphodiesterase inhibitor, T-440: possible management of eosinophilic inflammation by downregulation of interleukin-5 production. Int. Arch. Allergy Immunol. 111 (Suppl. 1), 16–18.

Kaminuma, O., Muurakami, S., Shinya, S., Matsunobu, K., Kikkawa, H., Matsubara, S., Toriumi, W., Ikezawa, K., Okudaira, H., Ito, K., 1997b. Late airway obstruction and neutrophil infiltration in sensitized mice after antigen provocation were suppressed by selective and non-selective phosphodiesterase inhibitors. Allergol. Int. 46, 101–108.

Kammer, G.M., 1988. The adenylate cyclase cAMP–protein kinase A pathway and regulation of the immune response. Immunol. Today 9, 222–229.

Kaneko, T., Alvarez, R., Ueki, I.F., Nadel, J.A., 1995. Elevated intracellular cyclic AMP inhibits chemotaxis in human eosinophils. Cell. Signalling 7, 527–534.

Kato, H., Araki, T., Itoyama, Y., Kogure, K., 1995. Rolipram, a cyclic AMP-selective phosphodiesterase inhibitor, reduces

neuronal damage following cerebral ischemia in the gerbil. Eur. J. Pharmacol. 272, 107–110.

Kawasaki, A., Hoshino, K., Osaki, R., Mizushima, Y., Yano, S., 1992. Effect of ibudilast: a novel anti-asthmatic agent on airway hypersensitivity in bronchial asthma. J. Asthma 29, 245–252.

Kay, A.B., 1985. Eosinophils as effector cells in immunity and hypersensitivity disorders. Clin. Exp. Immunol. 62, 1–12.

Kelly, J.J., Barnes, P.J., Giembycz, M.A., 1996. Phosphodiesterase 4 in macrophages: relationship between cAMP accumulation, suppression of cAMP hydrolysis and inhibition of [^{3}H] *R*-(−)-rolipram binding by selective inhibitors. Biochem. J. 318, 425–436.

Kips, J.C., Joos, G.F., Peleman, R.A., Pauwels, R.A., 1993a. The effect of zardaverine, an inhibitor of phosphodiesterase isoenzymes III and IV, on endotoxin-induced airway changes in rats. Clin. Exp. Allergy 23, 518–523.

Kips, J.C., Tavernier, J., Pauwels, R.A., 1992. Tumour necrosis factor causes bronchial hyperresponsiveness in rats. Am. Rev. Respir. Dis. 145, 332–336.

Kips, J.C., Tavernierm, J.H., Joos, G.F., Peleman, R.A., Pauwels, R.A., 1993b. The potential role of tumour necrosis factor-α in asthma. Clin. Exp. Allergy 23, 247–250.

Kita, H., Abu-Ghazaleh, R.I., Gleich, G.J., Abraham, R.T., 1991. Regulation of Ig-induced eosinophil degranulation by adenosine 3′,5′-cyclic monophosphate. J. Immunol. 146, 2712–2718.

Kleinman, E.F., Campbell, E., Giodano, L.A., Cohan, V.L., Jenkinson, T.H., Cheng, J.B., Shirley, J.T., Pettipher, E.R., Salter, E.D., Hibbs, T.A., Dicapua, F.M., Bordner, J., 1998. Striking effect of hydroxamic acid substitution on the phosphodiesterase type 4 (PDE4) and TNF-alpha inhibitory activity of two series of rolipram analogues: implications for a new active site model of PDE4. J. Med. Chem. 41, 266–270.

Koga, S., Morris, S., Ogawa, S., Liao, H., Bilezikian, J.P., Chen, G., Thompson, W.J., Ashikaga, T., Brett, J., Stern, D.M., Pinsky, D.J., 1995. TNF modulates endothelial properties by decreasing cAMP. Am. J. Physiol. 268, C1104–C1113.

Krause, D.S., Deutsch, C., 1991. Cyclic AMP inhibits IL-2 receptor expression in human T-cells: expression of both p55 and p75 subunits is affected. J. Immunol. 146, 2285–2296.

Kubota, S., 1996. The prolonged survival of eosinophils with IL-5 and its inhibition by increasing intracellular cAMP. Teikyo Med. J. 19, 563–570.

Kumari, M., Cover, P.O., Poyser, R.H., Buckingham, J.C., 1997. Stimulation of the hypothalamo-pituitary–adrenal axis in the rat by three selective type 4 phosphodiesterase inhibitors: in vitro and in vivo studies. Br. J. Pharmacol. 121, 459–468.

Lagente, V., Perrin, S., Mottin, G., Junien, J.L., Moodley, I., 1994. Type IV selective PDE IV inhibitors reduce eosinophil infiltration in the guinea-pig lung induced by antigen, platelet-activating factor and interleukin-5. Am. Respir. Crit. Care Med. 149, A762.

Lagente, V., Pruniaux, M.-P., Junien, J.-L., Moodley, I., 1995. Modulation of cytokine-induced eosinophil infiltration by phosphodiesterase inhibitors. Am. J. Respir. Crit. Care Med. 151, 1720–1724.

Lee, H.J., Koyano-Nakagawa, N., Naito, Y., Nishida, J., Arai, N., Arai, K.-I., Yokota, T., 1993. Cyclic AMP activates the IL-5 promoter synergistically with phorbol ester through the signaling pathway involving protein kinase A in mouse thymoma line EL-4. J. Immunol. 151, 6135–6142.

Lenhard, J.M., Kassal, D.B., Rocque, W.J., Hamacher, L., Holmes, W.D., Patel, I., Hoffman, C., Luther, M., 1996. Phosphorylation of a cAMP-specific phosphodiesterase (HSPDE4B2B) by mitogen-activated protein kinase. Biochem. J. 316, 751–758.

Lewis, G.M., Caccese, R.G., Heaslip, R.L., Bansbach, C.C., 1993. Effects of rolipram and CI-930 on IL-2 mRNA transcription in human Jurkat cells. Agents Actions 39, C89–C92.

Li, L., Yee, C., Beavo, J.A., 1999. CD3- and CD28-dependent induction of PDE7 required for T-cell activation. Science 283, 848–851.

Li, S.-H., Chan, S.C., Kramer, S.M., Hanifin, J.M., 1993. Modulation of leukocyte cyclic AMP phosphodiesterase activity by recombinant interferon-γ: evidence for a differential effect on atopic monocytes. J. Interferon Res. 13, 197–202.

Li, S.H., Chan, S.C., Toshitani, A., Leung, D.Y.M., Hanifin, J.M., 1992. Synergistic effects of interleukin-4 and interferon-gamma on monocyte phosphodiesterase activity. J. Invest. Dermatol. 99, 65–70.

Liang, L., Beshay, E., Prud'homme, G.J., 1998. The phosphodiesterase inhibitors pentoxifylline and rolipram prevent diabetes in NOD mice. Diabetes 47, 570–575.

Lidington, E., Nohammer, C., Dominguez, M., Ferry, B., Rose, M.L., 1996. Inhibition of the transendothelial migration of human lymphocytes but not monocytes by phosphodiesterase inhibitors. Clin. Exp. Immunol. 104, 66–71.

Lim, L.K., Hunt, N.H., Widemann, M.J., 1983. Reactive oxygen production, arachidonate metabolism and cyclic AMP in macrophages. Biochim. Biophys. Acta 114, 549–555.

Ma, D., Wu, P., Egan, R.W., Billah, M.M., Wang, P., 1999. Phosphodiesterase 4B gene transcription is activated by lipopolysaccharide and inhibited by interleukin-10 in human monocytes. Mol. Pharmacol. 55, 50–57.

Manning, C.D., McLaughlin, M.M., Livi, G.P., Cieslinski, L.B., Torphy, T.J., Barnette, M.S., 1996. Prolonged beta-adrenoceptor stimulation upregulates cAMP phosphodiesterase activity in human monocytes by increasing mRNA and protein for phosphodiesterases 4A and 4B. J. Pharmacol. Exp. Ther. 276, 810–818.

Masamune, H., Cheng, J.B., Cooper, K., Eggler, J.F., Marfat, A., Marshall, S.C., Shirley, J.T., Tickner, J.E., Umland, J.P., Vazquez, E., 1995. Discovery of micromolar PDE IV inhibitors that exhibit much reduced affinity for the [^{3}H] rolipram binding site: 3-norbornyl-4-methoxyphenylmethylene oxindoles. Bioorg. Med. Chem. Lett. 5, 965–968.

Matsubara, S., Fushimi, K., Kaminuma, O., Kikkawa, H., Ikezawa, K., Naito, K., 1997. Prevention of ozone-induced airway hyperresponsiveness and epithelial injury by phosphodiesterase inhibitors in guinea-pigs. Environ. Toxicol. Pharmacol. 3, 201–209.

McPhee, I., Pooley, L., Lobban, M., Bolger, G., Houslay, M.D., 1995. Identification, characterization and regional distribution in brain of RPDE6 (RNPDE4A5), a novel splice variant of the PDE4A cyclic AMP phosphodiesterase family. Biochem. J. 310, 965–974.

Michie, A.M., Lobban, M., Muller, T., Harnett, M.M., Houslay,

M.D., 1996. Rapid regulation of PDE2 and PDE4 cyclic AMP phosphodiesterase activity following ligation of the T cell antigen receptor on thymocytes: analysis using the selective inhibitors, erythro-9-(2-hydroxy-3-nonyl)-adenine (EHNA) and rolipram. Cell. Signalling 8, 97–110.

Michie, A.M., Rena, G., Harnett, M.M., Houslay, M.D., 1998. Upregulation of cAMP-specific PDE4 activity following ligation of the TCR complex on thymocytes is blocked by selective inhibitors of protein kinase C and tyrosyl kinases. Cell. Biochem. Biophys. 28, 161–185.

Miotla, J.M., Teixeira, M.M., Hellwell, P.G., 1998. Suppression of acute lung injury in mice by an inhibitor of phosphodiesterase 4. Am. J. Respir. Cell. Mol. Biol. 18, 411–420.

Molnar-Kimber, K., Yonno, L., Heaslip, R.J., Weichman, B.M., 1993. Modulation of TNF-α and IL-1β from endotoxin-stimulated monocytes by selective PDE isozyme inhibitors. Agents Actions 39, C77–C79.

Momose, T., Okubo, Y., Horie, S., Suzuki, J., Isobe, M., Sekiguchi, M., 1998. Effects of intracellular cyclic AMP modulators on human eosinophil survival, degranulation and CD11b expression. Int. Arch. Allergy Immunol. 117, 138–145.

Monaco, L., Vicini, E., Conti, M., 1994. Structure of two rat genes coding for a closely related rolipram-sensitive cyclic AMP phosphodiesterase. Multiple mRNA variants originate from alternative splicing and multiple start sites. J. Biol. Chem. 269, 347–357.

Montana, J.G., Cooper, N., Dyke, H., Oxford, J., Gristwood, R., Lowe, C., Kendall, H., Buckley, G., Maxey, R., Warneck, J., Gregory, J., Gowers, L., Galleway, F., Wills, R., Naylor, R., Tudhalar, B., Broadley, K., Danahay, H., 1999. A novel phosphodiesterase 4 (PDE4) inhibitor effects in cellular and animal models of asthma and early clinical trials. Am. J. Respir. Crit. Care Med. 159, A624.

Morandini, R., Ghanem, G., Portier-Lemarie, A., Robaye, B., Renaud, A., Boeynaems, J.M., 1996. Action of cAMP on expression and release of adhesion molecules in human endothelial cells. Am. J. Physiol. 270, H807–H816.

Mosmann, T.R., Cherwinski, H., Bond, M.W., Giedlin, M.A., Coffman, R.L., 1986. Two types of murine helper T-cell clone: I. Definition according to profiles of lymphokine activities and secreted proteins. J. Immunol. 136, 2348–2357.

Muller, T., Engels, P., Fozard, J.R., 1996. Subtypes of the type 4 cAMP phosphodiesterases: structure, regulation and selective inhibition. Trends Pharmacol. Sci. 17, 294–298.

Munoz, E., Zubiaga, A.M., Merrow, M., Sauter, N.P., Huber, B.T., 1990. Cholera toxin discriminates between T-helper 1 and 2 cell receptor-mediated activation: role of cyclic AMP in T-cell proliferation. J. Exp. Med. 172, 95–103.

Nakashima, S., Mizutani, T., Nakamura, Y., Takemura, S.O., Nakamura, M., Miyata, H., Katagiri, Y., Nozawa, Y., 1995. Effects of selective phosphodiesterase type IV inhibitor, rolipram, on signal transducing phospholipases in neutrophil: inhibition of phospholipases A2, D but not C. Toxicol. Endocrinol. 112C, 137–143.

Nemoz, G., Sette, C., Conti, M., 1997. Selective activation of rolipram-sensitive, cAMP-specific phosphodiesterase isoforms by phosphatidic acid. Mol. Pharmacol. 51, 242–249.

Newbold, P., Mather, A., Young, A., 1999. Effect of phosphodiesterase (PDE) inhibitors on active antigen-induced skin inflammation in sensitised guinea-pigs. Eur. Respir. J. 121, 71s.

Nielson, C.P., Vestal, R.E., Sturm, R.J., Heaslip, R., 1990. Effects of selective phosphodiesterase inhibitors on the polymorphonuclear leukocyte respiratory burst. J. Allergy Clin. Immunol. 86, 801–808.

Noel, P.E., Fletcher, J.R., Thompson, W.J., 1995. Rolipram and isoproterenol reverse platelet-activating-factor-induced increases in pulmonary microvascular permeability and vascular resistance. J. Surg. Res. 59, 159–164.

Norman, P., 1998. PDE4 inhibitors. Exp. Opin. Ther. Patents 8, 771–784.

Norman, P., 1999. Arofylline Almirall-Prodesfarma SA. Curr. Opin. Anti-Inflammatory Immunomodulatory Invest. Drugs 1, 48–50.

Novak, T.J., Rothenberg, E.V., 1990. Cyclic AMP inhibits induction of interleukin-2 but not of interleukin-4 in T-cells. Proc. Natl. Acad. Sci. U. S. A. 87, 9353–9357.

Nyman, U., Mussener, A., Larsson, E., Lorentzen, J., Klareskog, L., 1997. Amelioration of collagen II-induced arthritis in rats by the type IV phosphodiesterase inhibitor, rolipram. Clin. Exp. Immunol. 108, 415–419.

O'Connell, J.C., McCallum, J.F., McPhee, I., Wakefield, J., Houslay, E.S., Wishart, W., Bolger, G., Frame, M., Houslay, M.D., 1996. The SH3 domain of Src tyrosyl protein kinase interacts with the N-terminal splice region of the PDE4A cyclic AMP-specific phosphodiesterase RPDE6 (RNPDE4A5). Biochem. J. 318, 255–262.

Ohta, K., Sawamoto, S., Nakajima, M., Kubota, S., Tanaka, Y., Miyasaka, T., Nagai, A., Hirai, K., Mano, K., Miyashita, H., 1996. The prolonged survival of human eosinophils with interleukin-5 and its inhibition by theophylline via apoptosis. Clin. Exp. Allergy 26 (Suppl. 2), 10–15.

Okonogi, K., Gettys, T.W., Uhing, R.J., Tarry, W.C., Adams, D.O., Prpic, V., 1991. Inhibition of prostaglandin E_2-stimulated cyclic AMP accumulation by lipopolysaccharide in murine peritoneal macrophages. J. Biol. Chem. 266, 10305–10312.

Ollivier, V., Parry, G.C.N., Cobb, R.R., de Prost, D., Mackman, N., 1996. Elevated cyclic AMP inhibits NFκB-mediated transcription in human monocytic cells and endothelial cells. J. Biol. Chem. 271, 20828–20835.

Ortiz, J.L., Cortijo, J., Valles, J.M., Bou, J., Morcillo, E.J., 1992. Rolipram inhibits PAF-induced airway microvascular leakage in guinea-pig: a comparison with milrinone and theophylline. Fundam. Clin. Pharmacol. 6, 247–249.

Ortiz, J.L., Cortijo, J.M., Valles, J.M., Bou, J., Morcillo, E.J., 1993. Rolipram inhibits airway microvascular leakage induced by platelet-activating factor, histamine and bradykinin. J. Pharmacol. 45, 1090–1092.

Ortiz, J.L., Valles, J.M., Marticabrera, M., Cortijo, J., Morcillo, E.J., 1996. Effects of selective phosphodiesterase inhibitors on platelet-activating factor- and antigen-induced airway hyperreactivity, eosinophil accumulation, and microvascular leakage in guinea pigs. Naunyn-Schmiedeberg's Arch. Pharmacol. 353, 200–206.

Ozawa, Y., Yasui, S., Nagai, A., Konno, K., 1995. Protection and mechanism of bovine tracheal epithelial cell injury induced by H_2O_2–nicotinamide, niacin, theophylline and db-cAMP. Kokyu 14, 874–878.

Pahan, K., Khan, M., Singh, I., 1998. Therapy for X-adrenoleukodystrophy: normalization of very long chain fatty acids and inhibition of induction of cytokines by cAMP. J. Lipid Res. 39, 1091–1100.

Palfreyman, M.N., Souness, J.E., 1996. Phosphodiesterase type IV inhibitors. Prog. Med. Chem. 33, 1–52.

Park, D.J., Min, H.K., Rhee, S.G., 1992. Inhibition of CD3-linked phospholipase C by phorbol ester and by cAMP is associated with decreased phosphotyrosine and increased phosphoserine contents of PLCγ1. J. Biol. Chem. 27, 1469–1501.

Parronchi, P., Tiri, A., Macchia, D., De Carli, M., Biswas, P., Simonelli, C., Maggi, E., Del Prete, G., Ricci, M., Romagnani, S., 1990. Non-cognate contact-dependent B-cell activation can promote IL-4-dependent in vitro IgE synthesis. J. Immunol. 144, 2102–2108.

Patel, M.D., Samuelson, L.E., Klausner, R.D., 1987. Multiple kinases and signal transduction: phosphorylation of the T-cell antigen receptor complex. J. Biol. Chem. 262, 5831–5838.

Patrizio, M., Costa, T., Levi, G., 1995. Interferon-γ and lipopolysaccharide reduce cAMP responses in cultured glial cells: reversal by a type IV phosphodiesterase inhibitor. Glia 14, 94–100.

Pauwels, R.A., Kips, J.C., Paleman, R.A., Van Der Straeten, M.E., 1990. The effect of endotoxin inhalation on airway responsiveness and cellular influx in rats. Am. Rev. Respir. Dis. 141, 540–545.

Peachell, P.T., Undem, B.J., Schleimer, R.P., MacGlashan, D.W. Jr., Lichtenstein, L.M., Cieslinski, L.B., Torphy, T.J., 1992. Preliminary identification and role of phosphodiesterase isozymes in human basophils. J. Immunol. 148, 2503–2510.

Persson, C.G.A., 1988. Plasma exudation and asthma. Lung 166, 1–23.

Pincus, T., Callahan, L.F., 1993. What is the natural history of rheumatoid arthritis? Rheum. Dis. Clin. N. Am. 19, 123–151.

Pober, J.S., Slowik, M.R., De Luca, L.G., Ritchie, A.J., 1993. Elevated cyclic AMP inhibits endothelial cell synthesis and expression of TNF-induced endothelial leukocyte adhesion molecule-1, and vascular cell adhesion molecule-1, but not intercellular adhesion molecule-1. J. Immunol. 150, 5114–5123.

Probhakar, U., Lipshutz, D., Bartus, J.O., Slivjak, M.J., Smith, E.F., Lee, J.C., Esser, K.M., 1994. Characterization of cyclic AMP-dependent inhibition of LPS-induced TNF alpha production by rolipram, a specific phosphodiesterase IV (PDE IV) inhibitor. Int. J. Immunopharmacol. 16, 805–816.

Puurunen, J., Lucke, C., Schwabe, U., 1978. Effect of the phosphodiesterase inhibitor 4-(3-cyclopentyloxy-4-methoxyphenyl)-2-pyrrolidone (ZK 62711) on gastric secretion and gastric mucosal cyclic AMP. Naunyn-Schmiedeberg's Arch. Pharmacol. 304, 69–75.

Qian, Y., Girad, V., Martin, C.A.E., Molimard, M., Advenier, C., 1994. Rolipram, but not siguazodan or zaprinast, inhibits the excitatory non-cholinergic neurotransmission in guinea-pig bronchi. Eur. Respir. J. 7, 306–310.

Rabinovici, R., Feuerstein, G., Abdullah, F., Whiteford, M., Borboroglu, P., Sheikh, E., Phillip, D.-R., Ovadia, P., Bobroski, L., Bagasra, O., Neville, L.F., 1996. Locally produced tumour necrosis factor-α mediates interleukin-2-induced lung injury. Circ. Res. 78, 329–336.

Raeburn, D., Karlsson, J.-A., 1993. Effects of isoenzyme-selective inhibitors of cyclic nucleotide phosphodiesterase on microvascular leak in guinea-pig airways. J. Pharmacol. Exp. Ther. 267, 1147–1152.

Raeburn, D., Underwood, S.L., Lewis, S.A., Woodman, V.R., Battram, C.H., Tomkinson, A., Sharma, S., Jordan, R., Souness, J.E., Webber, S.E., Karlsson, J.-A., 1994. Anti-inflammatory and bronchodilator properties of RP 73401, a novel and selective phosphodiesterase type IV inhibitor. Br. J. Pharmacol. 113, 1423–1431.

Raeburn, D., Woodman, V., Buckley, G., Karlsson, J.A., 1991. Inhibition of PAF-induced microvascular leakage in the guinea-pig in vivo: the effects of rolipram and theophylline. Eur. Respir. J. 4, 590S, (Suppl. 14).

Rarnard, J.W., Serbert, A.F., Prasad, V.R., Smart, D.A., Strada, S.J., Taylor, A.E., Thompson, W.J., 1994. Reversal of pulmonary capillary ischemia–reperfusion injury by rolipram, a cyclic AMP phosphodiesterase inhibitor. J. Appl. Physiol. 77, 774–781.

Robichaud, A., Tattersall, P.D., Choudhury, I., Rodger, I.W., 1999. Emesis induced by inhibitors of type IV cyclic nucleotide phosphodiesterase (PDE IV) in the ferret. Neuropharmacology 38, 289–297.

Robicsek, S.A., Blanchard, D.K., Djeu, J.Y., Krzanowski, J.J., Szentivanyi, A., Polson, J.B., 1991. Multiple high-affinity cyclic AMP phosphodiesterases in human T-lymphocytes. Biochem. Pharmacol. 42, 869–877.

Rocque, W.J., Tian, G.C., Wiseman, J.S., Holmes, W.D., Zajacthompson, I., Willard, D.H., Patel, I.R., Wisely, G.B., Clay, W.C., Kadwell, S.H., Hoffman, C.R., Luther, M.A., 1997. Human recombinant phosphodiesterase 4B2B binds (*R*)-rolipram at a single site with two affinities. Biochemistry 36, 14250–14261.

Romagnani, S., 1991. Human Th-1 and Th-2: doubt no more. Immunol. Today 12, 256–257.

Ross, S.E., Williams, R.O., Mason, L.J., Mauri, C., Marinova-Mutafchieva, L., Malfait, A.-M., Maini, R.N., Feldmann, M., 1997. Suppression of TNF-α expression, inhibition of Th-1 activity, and amelioration of collagen-induced arthritis by rolipram. J. Immunol. 159, 6253–6259.

Sanjar, S., Aoki, S., Boubekeur, K., Chapman, I.D., Smith, D., Kings, M.A., Morley, J., 1990. Eosinophil accumulation in pulmonary airways of guinea-pigs induced by exposure to an aerosol of platelet activating factor: effect of anti-asthma drugs. Br. J. Pharmacol. 99, 267–272.

Santamaria, L.F., Palacios, J.M., Beleta, J., 1997. Inhibition of eotaxin-mediated human eosinophil activation and migration by the selective cyclic nucleotide phosphodiesterase type 4 inhibitor, rolipram. Br. J. Pharmacol. 121, 1150–1154.

Santing, R.E., Olymulder, C.G., Van der Molen, K., Meurs, H., Zaagsma, J., 1994. Phosphodiesterase inhibitors reduce bronchial hyperreactivity and airway inflammation in conscious, unrestrained guinea pigs. Am. J. Respir. Crit. Care Med. 149, A767.

Savany, A., Abriat, C., Nemoz, G., Lagarde, M., Prigent, A.-F., 1996. Activation of a cyclic nucleotide phosphodiesterase 4 (PDE4) from rat thymocytes by phosphatidic acid. Cell. Signalling 8, 511–516.

Sawiski, M.A., Rusin, L.J., Burns, T.L., Weinstein, G.D., Voorhees, J.J., 1979. Ro 20–1724: an agent that significantly improves psoriatic lesions in double-blind clinical trials. J. Invest. Dermatol. 73, 261–263.

Schad, F.U., Schudt, C., 1993. The specific types III and IV phosphodiesterase inhibitor, zardaverine, suppresses formation of tumour necrosis factor by macrophages. Eur. J. Pharmacol. 230, 9–14.

Schmidt, J., Hatzelmann, A., Fleibner, S., Heimann-Weitschat, I., Lindstaedt, R., Szelenyi, I., 1995. Effect of phosphodiesterase inhibition on IL-4 and IL-5 production of the murine TH2-type T cell clone, D10.G4.1. Immunopharmacology 30, 191–198.

Schmiechen, R., Schneide, H.H., Wachtel, H., 1990. Close correlation between behavioral response and binding in vivo for inhibitors of the rolipram-sensitive phosphodiesterase. Psychopharmacology 102, 17–20.

Schneider, H.H., Schmiechen, R., Brezinskim, M., Seidler, J., 1987. Stereospecific binding of the anti-depressant rolipram to brain-specific structures. Eur. J. Pharmacol. 127, 105–115.

Schudt, C., Tenor, H., Hatzelmann, A., 1995. PDE isoenzymes as targets for anti-asthma drugs. Eur. Respir. J. 8, 1179–1183.

Schwart, L.B., 1994. Mast cells: function and contents. Curr. Opin. Immunol. 6, 91–97.

Scott, A.I.F., Perini, A.F., Shering, P.A., Whalleym, L.J., 1991. In patient major depression: is rolipram as effective as amitriptyline? Eur. J. Clin. Pharmacol. 40, 127–129.

Sekut, L., Yarnall, D., Stimpson, S.A., Noel, L.S., Batemanfite, R., Clark, R.L., Brackeen, M.F., Menius, J.A., Connolly, K.M., 1995. Anti-inflammatory activity of phosphodiesterase (PDE) IV inhibitors in acute and chronic models of inflammation. Clin. Exp. Immunol. 100, 126–132.

Seldon, P.M., Barnes, P.J., Giembycz, M.A., 1998. Interleukin-10 does not mediate the inhibitory effect of PDE4 inhibitors and other cAMP-elevating drugs on lipopolysaccharide-induced tumors necrosis factor-α generation from human peripheral blood monocytes. Cell. Biochem. Biophys. 29, 179–201.

Seldon, P.M., Barnes, P.J., Meja, K., Giembycz, M.A., 1995. Suppression of lipopolysaccharide-induced tumor necrosis factor-α generation from human peripheral blood monocytes by inhibitors of phosphodiesterase 4: interaction with stimulants of adenylyl cyclase. Mol. Pharmacol. 48, 747–757.

Semmler, J., Wachtel, H., Endres, S., 1993. The selective type IV phosphodiesterase inhibitor rolipram suppresses tumour necrosis factor-α production by human mononuclear cells. Int. J. Immunopharmacol. 15, 409–413.

Sette, C., Conti, M., 1996. Phosphorylation and activation of a cAMP-specific phosphodiesterase by the cAMP-dependent protein kinase. Involvement of serine 54 in the enzyme activation. J. Biol. Chem. 271, 16526–16534.

Sette, C., Iona, S., Conti, M., 1994a. The short-term activation of a rolipram-sensitive, cyclic AMP-specific phosphodiesterase by thyroid-stimulating hormone in thyroid FRTL-5 cells is mediated by a cyclic AMP-dependent phosphorylation. J. Biol. Chem. 269, 9245–9252.

Sette, C., Vicini, E., Conti, M., 1994b. The rat PDE3/IVd phosphodiesterase gene codes for multiple proteins differentially activated by cyclic AMP-dependent protein kinase. J. Biol. Chem. 269, 18271–18274.

Seybold, J., Newton, R., Wright, L., Finney, P.A., Suttorp, N., Barnes, P.J., Adcock, I.M., Giembycz, M.A., 1998. Induction of phosphodiesterases 3B, 4A4, 4D1, 4D2, and 4D3 in Jurkat T-cells and in human peripheral blood T-lymphocytes by 8-bromo-cAMP and Gs-coupled receptor agonists. Potential role in β_2-adrenoreceptor desensitization. J. Biol. Chem. 273, 20575–20588.

Shakur, Y., Pryde, J., Houslay, M.D., 1993. Engineered deletion of the unique N-terminal domain of the cyclic AMP-specific phosphodiesterase RD1 prevents plasma membrane association and the attainment of enhanced thermostability without altering its sensitivity to inhibition by rolipram. Biochem. J. 292, 677–686.

Shichijo, M., Shimizu, Y., Hiramatsu, K., Inagaki, N., Tagaki, K., Nagai, H., 1997. Cyclic AMP-elevating agents inhibit mite-antigen-induced IL-4 and IL-13 release from basophil-enriched leukocyte preparation. Int. Arch. Allergy Immunol. 114, 348–353.

Smith, K.J., Scotland, G., Beattie, J., Trayer, I.P., Houslay, M.D., 1996. Determination of the structure of the N-terminal splice region of the cyclic AMP-specific phosphodiesterase RD1 (RNPDE4A1) by ^{1}H NMR and identification of the membrane association domain using chimeric constructs. J. Biol. Chem. 271, 16703–16711.

Soderling, S.H., Bayuga, S.J., Beavo, J.A., 1998a. Cloning and characterization of a cAMP-specific cyclic nucleotide phosphodiesterase. Proc. Natl. Acad. Sci. U. S. A. 95, 8991–8996.

Soderling, S.H., Bayuga, S.J., Beavo, J.A., 1998b. Identification and characterization of a novel family of cyclic nucleotide phosphodiesterases. J. Biol. Chem. 273, 15553–15558.

Soderling, S.H., Bayuga, S.J., Beavo, J.A., 1999. Isolation and characterization of a dual-substrate phosphodiesterase gene family: PDE10A. Proc. Natl. Acad. Sci. U. S. A. 96, 7071–7076.

Sommer, N., Loeschmann, P.-A., Northoff, G.H., Weller, M., Steinbrecher, A., Steinbach, J.P., Lichtenfels, R., Meyermann, R., Riethmuller, A., Fontana, A., Dichgans, J., Martin, R., 1995. The antidepressant rolipram suppresses cytokine production and prevents autoimmune encephalomyelitis. Nat. Med. 1, 244–248.

Sommer, N., Martin, R., McFarland, H.F., Quigley, L., 1997. Therapeutic potential of phosphodiesterase type 4 inhibition in chronic autoimmune demyelinating disease. J. Neuroimmunol. 79, 54–61.

Souness, J.E., Carter, C.M., Diocee, B.K., Hassall, G.A., Wood,

L.J., Turner, N.C., 1991. Characterization of guinea-pig eosinophil phosphodiesterase activity: assessment of its involvement in regulating superoxide generation. Biochem. Pharmacol. 42, 937–945.

Souness, J.E., Foster, M., 1998. Potential of phosphodiesterase inhibitors in the treatment of arthritis. Curr. Res. Rheum. Arthritis 2, 255–268.

Souness, J.E., Giembycz, M.A., 1994. Cyclic nucleotide phosphodiesterases in airways smooth muscle. In: Raeburn, D., Giembycz, M.A. (Eds.), Airways Smooth Muscle: Biochemical Control of Contraction and Relaxation. Birkhauser-Verlag, Basel, Switzerland, pp. 271–308.

Souness, J.E., Griffin, M., Maslen, C., Ebsworth, K., Scott, L.C., Pollock, K., Palfreyman, M.N., Karlsson, J.-A., 1996. Evidence that cyclic AMP phosphodiesterase inhibitors suppress TNF-α generation from human monocytes by interacting with a 'low-affinity' phosphodiesterase 4 conformer. Br. J. Pharmacol. 118, 649–658.

Souness, J.E., Houghton, C., Sardar, N., Withnall, M.T., 1997. Evidence that cyclic AMP phosphodiesterase inhibitors suppress interleukin-2 release from murine splenocytes by interacting with a 'low-affinity' phosphodiesterase 4 conformer. Br. J. Pharmacol. 121, 743–750.

Souness, J.E., Maslen, C., Webber, S., Foster, M., Raeburn, D., Palfreyman, M.N., Ashton, M.J., Karlsson, J.-A., 1995. Suppression of eosinophil function by RP 73401, a potent and selective inhibitor of cyclic AMP-specific phosphodiesterase: comparison with rolipram. Br. J. Pharmacol. 114, 39–46.

Souness, J.E., Rao, S., 1997. A proposal for pharmacologically distinct conformers of PDE4. Cell. Signalling 9, 227–236.

Souness, J.E., Scott, L.C., 1993. Stereospecificity of rolipram actions on eosinophil cyclic AMP-specific phosphodiesterase. Biochem. J. 291, 389–395.

Souness, J.E., Villamil, M.E., Scott, L.C., Tomkinson, A., Giembycz, M.A., Raeburn, D., 1994. Possible role of cyclic AMP phosphodiesterase in the actions of ibudilast on eosinophil thromboxane generation and airways smooth muscle tone. Br. J. Pharmacol. 111, 1081–1088.

Suda, Y., Tamura, G., Ohno, I., Maeda, K., Liu, Y., Yamauchi, K., Kurimoto, F., Shirato, K., 1998. Effects of phosphodiesterase inhibitors on secretions of human monokines. Allergol. Int. 47, 219–224.

Sullivan, G.W., Carper, H.T., Mandell, G.L., 1995. The specific type IV phosphodiesterase inhibitor rolipram combined with adenosine reduces tumor necrosis factor-α-primed neutrophil oxidative activity. Int. J. Immunopharmacol. 17, 793–803.

Sullivan, M., Egerton, M., Shakur, Y., Marquardson, A., Houslay, M.D., 1994. Molecular cloning and expression, in both COS-1 cells and *S. cerevisiae*, of a human cytosolic type IV (A), cyclic AMP-specific phosphodiesterase (hPDE(A-h6.1)). Cell. Signalling 6, 793–812.

Sunahara, R.K., Dessauer, C.W., Gilman, A.G., 1996. Complexity and diversity of mammalian adenylyl cyclases. Annu. Rev. Pharmacol. Toxicol. 36, 461–480.

Suttorp, N., Ehreiser, P., Hippensteil, S., Fuhrmann, M., Krull, M., Tenor, H., Schudt, C., 1996a. Hyperpermeability of pulmonary endothelial monolayer: protective role of phosphodiesterase isoenzymes 3 and 4. Lung 174, 181–194.

Suttorp, N., Hippensteil, S., Fuhrmann, M., Krull, M., Podzuweit, T., 1996b. Role of nitric oxide and phosphodiesterase isoenzyme II for reduction of endothelial hyperpermeability. Am. J. Physiol.: Cell Physiol. 39, C778–C785.

Suttorp, N., Hippensteil, S., Fuhrmann, M., Krull, M., Seybold, J., 1996c. Phosphodiesterase inhibition and pulmonary microvasculature — a new therapeutic approach? Atemwegs-Lungenkrankh. 22, 560–566.

Suttorp, N., Weber, U., Welsch, T., Schudt, C., 1993. Role of phosphodiesterases in the regulation of endothelial permeability in vitro. J. Clin. Invest. 91, 1421–1428.

Takei, K., Tokuyama, K., Kato, M., Morikawa, A., 1998. Role of cyclic adenosine monophosphate in reducing superoxide anion generation in guinea-pig alveolar macrophages. Pharmacology 57, 1–7.

Tamir, A., Isakov, N., 1994. Cyclic AMP inhibits phosphatidylinositol-coupled and -uncoupled mitogenic signals in T-lymphocytes. Evidence that cyclic AMP alters PKC-induced transcription regulation of members of the *jun* and *fos* family of genes. J. Immunol. 152, 3391–3399.

Teixeira, M.M., Miotla, J.M., Cooper, N., Gristwood, R.W., Hellewell, P.G., 1997. A comparison of the inhibitory activity of selective PDE4 inhibitors in eosinophil recruitment in guinea-pig skin. Memorias Do Instituto Oswaldo Cruz 92, 193–196.

Teixeira, M.M., Rossi, A.G., Giembycz, M.A., Hellewell, P.G., 1996. Effects of agents that elevate cyclic AMP on guinea-pig eosinophil homotypic aggregation. Br. J. Pharmacol. 118, 2099–2106.

Tenor, H., Hatzelmann, A., Church, M.K., Schudt, C., Shute, J.K., 1996. Effects of theophylline and rolipram on leukotriene C_4 (LTC_4) synthesis and chemotaxis of human eosinophils from normal and atopic subjects. Br. J. Pharmacol. 118, 1727–1735.

Tenor, H., Hatzelmann, A., Kupferschmidt, R., Stanciu, L., Djukanovic, R., Schudt, C., Wendel, A., Church, M.K., Shute, J.K., 1995a. Cyclic nucleotide phosphodiesterase isoenzyme activities in human alveolar macrophages. Clin. Exp. Allergy 25, 625–633.

Tenor, H., Stanciu, L., Schudt, C., Hatzelmann, A., Wendel, A., Djukanovic, R., Church, M.K., Shute, J.K., 1995b. Cyclic nucleotide phosphodiesterase from purified human CD_4 and CD_8 T-lymphocytes. Clin. Exp. Allergy 25, 616–624.

Torphy, T.J., 1998. Phosphodiesterase isozymes: molecular targets for novel anti-asthma agents. Am. J. Respir. Crit. Care Med. 157, 351–370.

Torphy, T.J., Stadel, J.M., Burman, M., Cieslinski, L.B., McLaughlin, M.M., White, J.R., Livi, G.P., 1992a. Coexpression of human cyclic AMP-specific phosphodiesterase activity and high-affinity rolipram binding in yeast. J. Biol. Chem. 267, 1798–1804.

Torphy, T.J., Undem, B.J., 1991. Phosphodiesterase inhibitors: New opportunities for the treatment of asthma. Thorax 46, 512–523.

Torphy, T.J., Zhou, H.-L., Cieslinski, L.B., 1992b. Stimulation of beta-adrenoceptors in a human monocyte cell line (U937) upregulates cyclic AMP-specific phosphodiesterase activity. J. Pharmacol. Exp. Ther. 263, 1195–1205.

Torphy, T.J., Zhou, H.L., Foley, J.J., Sarau, H.M., Manning,

C.D., Barnette, M.S., 1995. Salbutamol upregulates PDE4 activity and induces a heterologous desensitization of U937 cells to prostaglandin E_2. Implications for the therapeutic use of β-adrenoceptor agonists. J. Biol. Chem. 270, 23598–23604.

Townley, R.G., 1993. Elevated cyclic AMP-phosphodiesterase in atopic disease: cause or effect? J. Lab. Clin. Med. 121, 15–17.

Treese, N., Rhein, S., 1990. Phosphodiesterase inhibitors: Clinical experience. Drugs News Perspect. 3, 99–105.

Tsurata, L., Lee, H.-J., Masuda, E.S., Koyano-Nakagawa, N., Arai, N., Arai, K., Yokota, T., 1995. Cyclic AMP inhibits expression of the IL-2 gene through the nuclear factor of activated T cells (NF-AT) site, and transfection of NF-AT cDNAs abrogates the sensitivity of EL-4 cells to cyclic AMP. J. Immunol. 154, 5255–5264.

Turner, C.R., Andersen, C.J., Smith, W.B., Watson, J.W., 1994. Effects of rolipram on responses to acute and chronic antigen exposure in monkeys. Am. J. Respir. Crit. Care Med. 149, 1153–1159.

Turner, C.R., Cohan, V.L., Cheng, J.B., Showell, H.J., Pazoles, C.J., Watson, J.W., 1996. The in vivo pharmacology of CP-80,633, a selective inhibitor of phosphodiesterase 4. J. Pharmacol. Exp. Ther. 278, 1349–1355.

Turner, N.C., Wood, L.J., Burns, F.M., Gueremy, T., Souness, J.E., 1993. The effect of cyclic AMP and cyclic GMP phosphodiesterase inhibitors on the superoxide burst of guinea-pig peritoneal macrophages. Br. J. Pharmacol. 108, 876–883.

Uchiyama-Tsuyuki, Y., Okuyama, S., Araki, H., 1996. VA-045, a novel apovincaminic acid derivative, attenuates neuronal injury induced by hypoxia or by excitatory amino acids in cultures of rat cortices. Life Sci. 59, 1571–1578.

Ukena, D., Rentz, K., Reiber, C., Sybrecht, G.W., 1995. Effects of the mixed phosphodiesterase III/IV inhibitor, zardaverine, on airway function in patients with chronic airflow obstruction. Respir. Med. 89, 441–444.

Undem, B.J., Meeke, S.N., Chen, J., 1994. Inhibition of neurally mediated non-adrenergic, non-cholinergic contractions of guinea-pig bronchus by isozyme-selective phosphodiesterase inhibitors. J. Pharmacol. Exp. Ther. 271, 811–817.

Underwood, D.C., Bochnowicz, S., Osborn, R.R., Kotzer, C.J., Luttmann, M.A., Hay, D.W.P., Gorycki, P.D., Christensen, S.B., Torphy, T.J., 1998. Antiasthmatic activity of the second-generation phosphodiesterase 4 (PDE4) inhibitor SB 207499 (Ariflo) in the guinea-pig. J. Pharmacol. Exp. Ther. 287, 988–995.

Underwood, D.C., Kotzer, C.J., Bochnowicz, S., Osborn, R.R., Luttmann, M.A., Hay, D.W.P., Torphy, T.J., 1994. Comparison of phosphodiesterase III, IV and dual III/IV inhibitors on bronchospasm and pulmonary eosinophil influx in guinea-pigs. J. Pharmacol. Exp. Ther. 270, 250–259.

Underwood, D.C., Matthews, J.K., Osborn, R.R., Bochnowicz, S., Torphy, T.J., 1997. The influence of endogenous catecholamines on the inhibitory effects of rolipram against early- and late-phase response to antigen in the guinea-pig. J. Pharmacol. Exp. Ther. 280, 210–219.

Underwood, D.C., Osborn, R.R., Novak, L.B., Matthews, J.K., Newsholme, S.J., Undem, B.J., Hand, J.M., Torphy, T.J., 1993. Inhibition of antigen-induced bronchoconstriction and eosinophil infiltration in the guinea-pig by the cyclic AMP-specific phosphodiesterase inhibitor, rolipram. J. Pharmacol. Exp. Ther. 266, 306–313.

Uno, T., Tanaka, H., Nagai, H., 1998. Effects of isoenzyme selective phosphodiesterase inhibitors on bacterial lipopolysaccharide-induced bronchial hyperreactivity in guinea-pig. Gen. Pharmacol. 30, 167–173.

Valette, L., Prigent, A.F., Nemoz, G., Anker, G., Macovschi, O., Lagarde, M., 1990. Concanavalin A stimulates the rolipram-sensitive isoforms of cyclic nucleotide phosphodiesterase in rat thymic lymphocytes. Biochem. Biophys. Res. Commun. 169, 864–872.

Van der Poow-Kraan, T., Van Kooten, C., Rensink, I., Aarden, L., 1992. Interleukin (IL)-4 production by human T-cells: differential regulation of IL-4 vs. IL-2 production. Eur. J. Immunol. 22, 1237–1241.

Van Tits, L.J.H., Michel, M.C., Motulsky, H.J., Maisel, A.S., Brodde, O.-E., 1991. Cyclic AMP counteracts mitogen-induced inositol phosphate generation and increases in intracellular Ca^{2+} concentrations on human leukocytes. Br. J. Pharmacol. 103, 1288–1294.

Van Wauwe, J., Aerts, F., Walter, H., de Boer, M., 1995. Cytokine production by phytohemagglutinin-stimulated human blood cells: effects of corticosteroids, T-cell immunosuppressants and phosphodiesterase IV inhibitors. Inflammatory Res. 44, 400–405.

Verghese, M.W., McConnell, R.T., Lenhard, J.M., Hamacher, L., Jin, S.-L.C., 1995a. Regulation of distinct cyclic AMP-specific phosphodiesterase (phosphodiesterase type 4) isozymes in human monocytic cells. Mol. Pharmacol. 47, 1164–1171.

Verghese, M.W., McConnell, R.T., Strickland, A.B., Gooding, R.C., Stimpson, S.A., Yarnell, D.P., Taylor, J.D., Furdon, P.J., 1995b. Differential regulation of human monocyte-derived TNF-α and IL-1β by type IV cAMP-phosphodiesterase (cAMP-PDE) inhibitors. J. Pharmacol. Exp. Ther. 272, 1313–1320.

Vicini, E., Conti, M., 1997. Characterization of an intronic promoter of a cyclic adenosine 3′,5′-monophosphate (cAMP)-specific phosphodiesterase gene that confers hormone and cAMP inducibility. Mol. Endocrinol. 11, 839–850.

Wancio, D., Damle, N.K., Bansbach, C.C., 1995. Pharmacological inhibition of CD28-stimulated T-cell activation. Inflammatory Res. 44 (Suppl. 2), S203–S204.

Wang, P., Wu, P., Siegel, M.I., Egan, R.W., Billah, M.M., 1995. Interleukin (IL)-10 inhibits nuclear factor κB (NFκB) activation in human monocytes. IL-10 and IL-4 suppress cytokine synthesis by different mechanisms. J. Biol. Chem. 270, 9558–9563.

Weston, M.C., Anderson, N., Peachell, P.T., 1997. Effects of phosphodiesterase inhibitors on human lung mast cell and basophil function. Br. J. Pharmacol. 121, 287–295.

Weston, M.C., Peachell, P.T., 1998. Regulation of human mast cell and basophil function by cAMP. Gen. Pharmacol. 31, 715–719.

Wiemer, G., Hellwich, U., Wellstein, A., Dietz, J., Hellwich, M., Palm, D., 1982. Energy-dependent extrusion of cyclic 3′,5′-adenosine monophosphate. A drug-sensitive regulatory mecha-

nism for the intracellular nucleotide concentration in rat erythrocytes. Naunyn-Schmiedeberg's Arch. Pharmacol. 321, 239–246.

Wilson, M., Sullivan, M., Brown, N., Houslay, M.D., 1994. Purification, characterization and analysis of rolipram inhibition of a human type IV(A) cyclic AMP-specific phosphodiesterase expressed in yeast. Biochem. J. 304, 407–415.

Wright, C.D., Kuipers, P.J., Kobylarz-Singer, D., Devall, L.J., Klinkefus, B.A., Weishaar, R.E., 1990. Differential inhibition of neutrophil functions: role of cyclic AMP-specific, cyclic GMP-insensitive phosphodiesterase. Biochem. Pharmacol. 40, 699–707.

Wright, L.C., Seybold, J., Robichaud, A., Adcock, I.M., Barnes, P.J., 1998. Phosphodiesterase expression in human epithelial cells. Am. J. Physiol. 275, L694–L700.

Xiang, M., Zaccone, P., Dimarco, R., Magro, G., Dimauro, M., Beltrami, B., Meroni, P.L., Nicoletti, F., 1999. Prevention by rolipram of concanavalin A-induced T-cell-dependent hepatitis in mice. Eur. J. Pharmacol. 367, 399–404.

Yamashita, N., Hayashi, A., Baba, J., Sawa, A., 1997. Rolipram, a phosphodiesterase-4-selective inhibitor, promotes the survival of cultured rat dopaminergic neurons. Jpn. J. Pharmacol. 75, 155–159.

Yoshikawa, M., Suzumura, A., Tamaru, T., Takayanagi, T., Sawada, M., 1999. Effects of phosphodiesterase inhibitors on cytokine production by microglia. Mult. Scler. 5, 126–133.